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Thesis

THE REACTIONS OF NORMAL SKIN TO ULTRAVIOLET RADIATIONS

by

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INTRODUCTION

Ultraviolet light has been the subject of voluminous research concerning its various effects, both harmful and beneficial. In recent years ultraviolet radiations have become very popular in therapeutics as a means of alleviating and curing numerous organic disorders; and, as a result, the increasing interest in this field has led to the publication of thousands of papers on the physical characteristics and physiological effects of ultraviolet light. Studies have been made concerning its effectiveness in killing bacteria and unicelluler organisms, in increasing and retarding the growth of cells, as a therapeutic aid in tuberculosis, for sterilization of objects and processes such as surgical instruments and hospital wards, and in many other fields that are too numerous to mention here. However, in order to understand its uses, one must have first a thorough knowledge of the primary physiological reactions that occur upon administration of ultraviolet to the organism.

Because of the great interest in the effect of ultraviolet on man, this paper is mainly concerned with human reactions to ultraviolet radiations. In addition, the author has found it necessary to limit this paper to the normal reactions, as abnormal reactions in themselves would comprise another extensive review of the literature and, thus could not be treated adequately here. The effects of ultraviolet application vary from the immediate reactions in the skin to more remote ones occurring in deeper body organs and systems. Because the skin plays a very import-

ent role in determining to what extent the ultraviolet rays will penetrate into the body and, too, because the skin is the first of the body parts to react to radiations, the subject of this thesis has been built around the physiological effects of ultraviolet on normal skin. These effects usually exhibit themselves as sunburn and suntan; and, this being the case, the greater portion of this paper is concerned with those skin reactions which ultimately lead to the visible manifestation of sunburn which is normally followed by its counterpart, suntan.

It is also necessary to consider the antipolitic action of ultraviolet, since this reaction occurs in the superficial layers of the epidermis, and is one of the first reactions to occur after exposure to radiations. Furthermore, ultraviolet has been reported as one of the main causes of cutaneous cancer; and, although this type of cancer does not appear until after many years of constant exposure, I think that it is a very pertinent aspect of this thesis.

I wish to express my deepest gratitude to Professor George Fuller for his sincere interest and unselfish aid without which completion of this paper would hardly have been possible.

Mechanics of ultraviolet light

In order to have a thorough understanding of the effects of ultraviolet radiation, it is first necessary to have some idea of the nature of this type of radiation and of the fundamental laws governing its mechanics.

Blum(1941) has coined the term "photodynamic action" to describe the property of light which produces various chemical reactions in objects exposed to light. The phenomenon involving the sensitization of substances by the photodynamic action is called "photosensitization". The sensitizing substances absorb the light by atoms or molecules which, while remaining unchanged in themselves, cause reactions of other components, which are not sensitive to light, by transformation of their energy through collision or some other means. The radiation necessary to initiate the photosensitive chemical processes resulting from photodynamic action must fall within the absorption range of the particular sensitizer involved. This idea follows the Grotthus-Draper Law, which is the first law of photochemistry, that only those wave-lengths of light which are absorbed by a system may produce photochemical reactions in that system.

The wave-lengths of ordinary sunlight of greatest significance in photogiology extend, approximately, in a region from about 2,500 to 18,500 Angstrom units (A.U.). The effect of wave-lengths within the range of 3,300 A.U. to 4,500 A.U. are of most concern in the present study since this is the portion of the spectrum which contains the ultraviolet light. The absorption of protoplasm is greatly increased within this range so

that the penetration of these waves into the body is very slight. As a result, the direct effects of this short wave-length radiation is very superficial, causing any bodily changes to occur only in the area of the skin. It is an important fact to remember that the amount of light absorbed is a function of the number of absorbing particles and, hence, of the thickness of the system through which the radiation is directed. For this reason, ultraviolet radiation only penetrates a very short distance into the skin, and thus does not affect tissues at a considerable depth. These wave-lengths are much shorter than those involved in human vision, which vary between 4,000 and 6,800 A.U. For purposes of measurements, protection, and other needs, these shorter waves can be filtered out by ordinary window glass.

The action spectrum is the curve relating wave-length and effectiveness in producing a given biological response. It follows rather closely the absorption spectrum of the substance whose molecules serve as the light absorber for that particular process. The limits of an action spectrum probably correspond to the limits of an absorption band of the light absorbing substance; consequently, some degree of correspondence between absorption spectrum and action spectrum is to be anticipated. Although action spectra and absorption spectra may not agree completely, a maximum of action is not to be expected in a region of minimum absorption. Two or more photobiological processes having similar action spectra may have the same light absorbers.

Because of the high incidence of destructiveness, it may be

generalized that ultraviolet radiation shorter than 3,300 A.U. is, as a rule, destructive to living systems. It is lethal to microscopic organisms; it causes sunburn in man, and in other large animals it produces effects varying from mild irritation to severe surface lesions. The ultraviolet wave in itself is not destructive. The destructive action is attributed to the fact that important photolabile components of protoplasm show specific absorption below wave-lengths of 3,300 A.U. so that most cells are vulnerable to such radiation.

The light absorbers for photobiologic reactions

The true nature of the light absorbing substance of the skin has yet to be determined. However, Henri(1912) pointed out the similarity between the action spectra of ultraviolet light and the absorption spectra of proteins in that the maxima occur at the same spectral region for both, which is around 2,800 A.U. This observation does not appear to be important since many substances absorb characteristically in this spectral region, particularly benzoid ring compounds. However, it is important because proteins are very basic constituents of the cell and are readily altered by ultraviolet radiation. Proteins must be considered as possible light absorbers. On the other hand, nucleic acids are also important cellular constituents and also absorb in the same spectral region as proteins. Consequently, nucleic acids may be the absorbing substances. But, Caspersson(1936) determined the absorption spectra for both sodium-thymo-nucleic acid and the protein, serum albumin. He

demonstrated, as indicated in Figure 1., that the protein more closely resembles epidermal absorption of waves below 3,300 A.U. The differences in the height of the two curves are quantitative differences of absorption at the maxima, but this may be disregarded in making comparison with action spectra, since the latter provides only relative values. The important difference between the absorption spectra of the nucleic acid and that of the protein is the fact that the maximum for the protein occurs at 2,800 A.U. which is just about the same as the maximum for the epidermis, whereas the maximum for the nucleic acid is at 2,600 A.U. which is considerably below the maximum for the epidermis. Another important difference is that protein absorption increases more rapidly than nucleic acid toward the shorter wave-lengths, as does the epidermis.

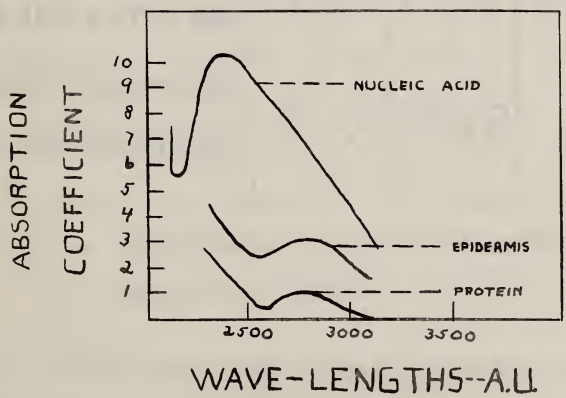

Figure 1. Absorption spectra of a nucleic acid and a protein. Caspersson (1936).

Quantitative absorption of ultraviolet light

The first quantitative measurements of absorption and transmission of ultraviolet light through the skin were made by Hasselbalch (1911), who gave figures as to the extent to which light of the different ultraviolet wave-lengths penetrate the skin. Hasselbalch claimed that the depth of the penetration of the shorter ultraviolet rays through the skin is, for the most part, not more than 0.1 mm. This figure has been confirmed by Henri (1912), Glitscher (1919), and, most recently, by Laurens (1939). The following table summarizes the results as obtained by Hasselbalch.

	WAVE-LENGTHS---A.U.							
	4360	4050	3660	3540	3130	3020	2970	2890
PERCENT TRANSMITTED BY SKIN 0.1 M.M. THICK	59	55	49	42	30	8	2	.01
PERCENT TRANSMITTED BY SKIN 1.0 M.M. THICK	.5	.3	.08	.02				

Table 1. Transmission of light through the skin.
Hasselbalch (1911).

The above figures conclusively indicate, according to Hasselbalch, that light less than 3,000 A.U. is absorbed by the epidermis in a layer 0.1 mm. thick; and, as the wave-lengths become increasingly shorter, the smaller the layer of epidermis required to completely absorb them. Also, as the epidermal layers

become thicker, a decreasing amount of light is able to penetrate through them. This latter fact is important to keep in mind; for, it is pointed out in a later discussion that the skin thickens and protects lower layers from ultraviolet rays.

However, all investigators have not been in complete agreement with the conclusions reached by Hasselbalch and the others who confirmed him. Macht, Anderson, and Bell (1928) claimed that the above results are not valid because dead skin was used in the experiments. They maintain that, in dead skin, the protoplasm differs not only biologically but in physical and chemical properties from living protoplasm. In their experiments, Macht, Anderson, and Bell used the skin of a living anaesthetised rabbit and reflected the monochromatic light of a mercury vapor arc lamp from its body. The energy transmitted through the skin was recorded by a thermopile and a galvanometer. As a result, they found that the penetration of ultraviolet rays through living skin is much greater than had hitherto been supposed, with some of the shorter rays penetrating deeper than the longer rays. They showed that between the wave-lengths of 3,600 A.U. and 3,800 A.U. the percentage of transmission increased from 11.4% to 56.3% and that there was then a drop to 23% transmission at 2,650 A.U. with a further rise to 4[illegible].8% transmission at 2,537 A.U. They also pointed out that white human skin is more permeable to irradiations than negro skin on account of the pigment present in the latter.

As can be seen, these results of Macht, Anderson and Bell are in direct opposition to those conclusions reached by Hassel-

balch, Henri, Glitcher, and Laurens. Whereas the latter investigators claim that the percentage of transmission through the skin decreases with shorter ultraviolet wave-lengths, the former hold that the percentage of transmission is greater for some of the shorter ultraviolet wave-lengths than for longer waves and that these differences in results are due to the use of living skin in their experiments.

Other investigators are not in accord with the results reported by Macht, Anderson and Bell. For example, Hill (1928) criticizes their conclusion that skin 1.175 mm. thick transmits 56.3% of the ultraviolet rays at 2,800 A.U. and 42.8% at 2,537 A.U. but only 11.4% at 3,600 A.U. As pointed out, these results are wholly at variance with those of Hasselbalch and the other early investigators who claim that the short ultraviolet rays can not pass through more than 0.1 mm. of skin. Hill's own physiological experiments show that such substances as protein serum protect tubercle bacilli, a screen of omentum protects paramecia, and rabbit's skin protects human skin from erythema. All of these substances are very thin and yet they protect the living protoplasm below them from the ultraviolet rays. These biologic tests confirm the view that the ultraviolet rays of the active region of 3,000 A.U. are absorbed by the epidermis alone when this is 0.5 mm. thick, so that any effective biologic action of the mercury vapor lamp on deeper tissues is prevented even with exposures of from one to two hours. With an exposure of five minutes, effective action on deeper tissues will be screened off by the epidermis even when it is much thinner

than 0.5 mm. Thus, Hill has also confirmed the results of the earlier investigators by demonstrating that the shorter ultraviolet rays are unable to penetrate through very thin layers of the skin, and opposes the view of Macht, Anderson, and Bell that a considerable amount of the shorter ultraviolet wave-lengths is able to penetrate through skin as much as 1.175 mm. thick. However, it must be pointed out that Hill, too, used dead skin in his experiments, despite the view of Macht, Anderson, and Bell that dead skin markedly differs in many respects from the live skin used in their experiment and, thus, the results will be greatly altered because of the two different types of media used in conducting the experiments.

Bachem (1928) also criticizes the paper of Macht, Anderson, and Bell on the grounds that the figures reached by these experimenters, on the transmission of ultraviolet light through the skin, are too high due to the fact that stray light rays were also measured on the thermopile as well as the incident rays and that by eliminating these scattered rays, the error will be corrected and the right figures will result. These stray rays are caused by the scattering of the incident rays over the surface of the epidermis and fluorescence of the light that has been absorbed by the tissues. Bachem claimed that by devising some means of eliminating these scattered light rays only the pure absorption of the light by the epidermis will be measured and lower figures as to the amount of transmission will be the result.

Anderson and Hacht (1928) conducted further experiments in order to answer the above criticisms of Hill and Bachem. In deference to Bachem, they used a special photometer to control the thermopile method but they found that the amount of stray light involved was negligible to the results obtained. They admitted the error due to fluorescence of the skin and devised means to correct against it. Nevertheless, despite these additional precautions, their findings still showed a rise of transmission through skin 1.2 mm. thick with the shorter ultraviolet wave-lengths although the increase was not as great as observed in their previous experiment. They showed a rise of transmission from 7.5% to 9.8% between the wave-lengths of 3,020 A.U. and 2,800 A.U. These results are still contrary to the findings of previous workers on the ultraviolet absorption of other protein substances. However, Anderson and Hacht again emphasized that this difference was due to the fact that they used live tissues whereas the previous workers used dead tissues. They again claimed that it is very important to use live tissues in the study of biologic light reaction.

Bachem and Kuntz (1928) finally conducted experiments to determine if there actually was any difference between the absorption of live and dead tissue in these studies on the penetration of ultraviolet light through the epidermis. The results of their investigations convincingly indicate that the difference between living tissue and dead tissue kept in Ringer solution and on ice for one or two days is smaller than can be measured exactly. They also found that the penetration of ultra-

violet light is greater than given in the older literature, particularly by Hasselbalch (1911), and smaller than suggested by Macht and his co-workers (1928). Bachem and Reed (1929) continued their experimentation on this problem and their results still showed that little difference exists between live and dead animal tissue in transmission, for the next few hours after death, if kept wet in Ringer's solution and well stretched. Therefore, with proper precautions, dead tissue can be used for the study of light transmission through animal skin. They further noted that the pronounced difference in transmission through dried and wet skin permitted estimation of the relative importance of true absorption and scattering. This was an urgent problem because measurement of absorption of radiation by skin is very difficult due to the fact that much incident light is scattered through the skin and this scattered light is not measured. The only light measured is that leaving the skin at right angles to its surface and this results in absorption values that are too high. As a result of their experiment, Bachem and Reed pointed out that only the true absorption coefficient changed strongly with the wave-length whereas the scattering coefficient was nearly constant. This meant that once the amount of scattering through a piece of tissue was determined this figure could be used at all times, no matter what wave-length was employed, to find the amount of transmission through the skin.

Lucas (1931) attempted to estimate the true absorption of epidermis by clearing the tissue with glycerol, which in no way altered the absorption of the epidermis, and making use of both

the scattered and incident radiation used. The absorption spectra determined by Lucas show a maximum at about 2,700-2,800 A.U. with a minimum at about 2,500-2,600 A.U., an increasing absorption toward the shorter wave-lengths, and extremely little absorption by wave-lengths longer than 3,300 A.U. This absorption spectrum is characteristic of many proteins, as shown above. He accounts for protein absorption in this region as probably due to benzenoid amino-acid structures, and the absorption spectra of tyrosine and tryptophane closely resemble the protein absorption spectra. It is quite probable that the principal absorption of sunburn radiation by the epidermis is due to proteins containing benzenoid amino-acid structures in their configuration, although other substances may contribute significantly.

The results of Lucas also show a 1.5 to 30 times greater amount of transmitted light recorded for wave-lengths 4,000-2,890 A.U. than do those of Hasselbalch. This difference is illustrated in the following figure. He attributes this discrepency as probably due to a failure on Hasselbalch's part to differentiate between the loss of transmitted light by scattering, which loss is progressively greater as the wave-length decreases, and the loss by true absorption. This scattering, while causing great diminution of the intensity of the light received by the spectrograph, causes much less interference in the transmission of the light to underlying tissues when skin is irradiated in vivo. As a result, for light of wave-length 3,000 A.U. and less, the effect of scattering is dwarfed by true absorption of the epi-

dermis.

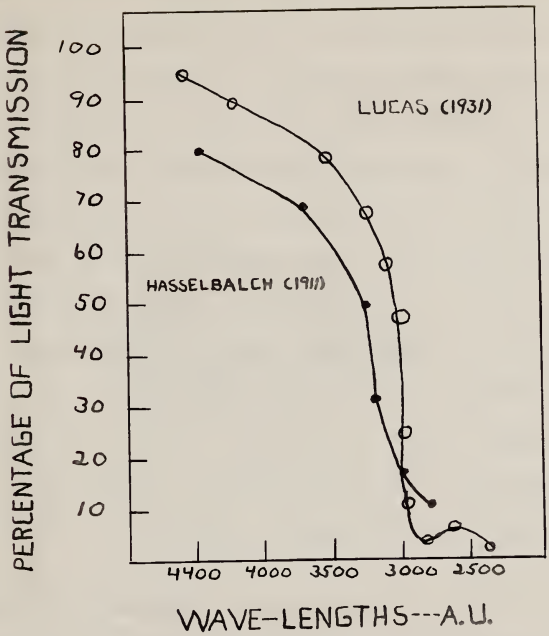

Figure 2. Comparison between Lucas and Hasselbalch of light transmission through the skin. Lucas (1931).

Thus, we see that it is difficult to make comparisons between measurements because of different methods used by the different investigators and the thickness of the various layers of the skin is not the same for different regions of the body surface. This factor is important because the experimenters, indiscriminately using skin from different parts of the body, will, most naturally, arrive at figures which do not agree with those of other findings since the thickness of the skin plays an extremely important role in the amount of absorption involved.

Another salient factor is that the individual layers of the same piece of skin are not of uniform thickness and this will also have an effect on the final results obtained. Therefore, it is impossible to make more than a rough estimate of the depth of penetration of ultraviolet radiation. However it can be concluded generally, on the basis of facts which will be borne out in experiments to be presented later, that very few waves shorter than 2,900 A.U. reach the Malpighian layer and none reach the corium. Wave-lengths shorter than 2,900 A.U. produce erythema, which is the microscopic dilation of blood vessels in the corium; but since they do not penetrate to these vessels, the erythema is not a direct effect. Longer waves of 3,000-3,300 A.U. may reach the corium but do not get to the subcutaneous tissue.

Histology of the skin

Before proceeding further with this review of the effect of ultraviolet waves on the skin, a discussion of the histology of the skin is pertinent. The outer layer of the epidermis is the stratum corneum which is made up of non-living material derived from layers lying beneath. Below the corneum is the stratum granulosum which is a narrow granular layer consisting of a few scattered masses of cells. Beneath the stratum granulosum is the stratum germinativum made up of the stratum spinosum, or prickle cell layer, and the basal cell layer, which is a wavy line of cells with darker nuclei lying below the stratum spinosum. The stratum germinativum is also known as the mucosum or the Malpighian layer. The basal cell layer is the

region of the epidermis where new cells are being formed by division and will degenerate subsequently to become the cornium. Below the epidermis is the corium, or dermis. Here are found tiny projections called dermal papillae which contain the superficial blood vessels. This is the papillary layer of the corium and it overlies the reticular layer of the corium. Finally, beneath the corium is found the subcutaneous tissue.

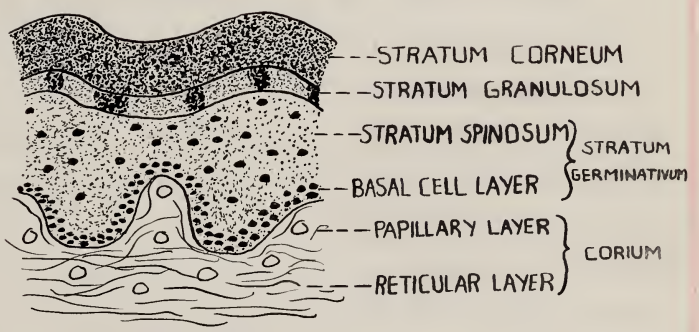

Figure 3. Histological section of the skin.
Maximov and Bloom (1940).

Qualitative absorption of ultraviolet light

Heretofore, the quantitative absorption of the ultraviolet light, as determined by the thickness of the epidermis, has been presented. The first qualitative differences in absorption of ultraviolet light by the various layers of the epidermis were noted by Dachem and Kuntz (1928) while they were conducting their experiments on the absorption differences of live and dead animal tissue. They observed that the various layers of skin exert a very different absorption and each of these layers show

characteristic selective absorption bands. However, at this time they did not continue along this line of investigation. Finally, Eschem (1929,1930), Eschem and Kuntz (1929), and Eschem and Wood (1930, 1931) resumed studies on the absorption differences of the various layers of the skin. They calculated the absorption coefficients of the different skin layers from the amount of ultraviolet light transmitted by these layers. The absorption curves showed marked differences for the various layers. Eschem (1930) claimed that the absorption coefficient does not hold accurately for various thicknesses of sections on account of reflection, scattering, and fluorescence. The skin cannot be considered as consisting of a series of layers with identical absorption as each layer shows a pronounced absorption difference. The investigations by these men show that there is a greater variation in percentage penetration of ultraviolet than in other parts of the light spectrum. They found that most of the visible light was absorbed in the corium; the longer rays penetrated into the subcutaneous layers. The waves not quite as short as the ultraviolet were absorbed chiefly in the Malpighian layers and somewhat in the corium. At a wave-length of 2,750 A.U. practically all of the radiation was absorbed in the stratum corneum and the stratum granulosum. On both sides of this band, near 3,000 A.U. and 2,500 A.U., the penetration is greater with a large amount of radiant energy reaching the stratum germinativum and the corium. It is here, in the layers of the stratum germinativum and the corium, that the erythema originates under the shadow of the upper layers. The stratum

corneum and the stratum granulosum must play an important role in the light protection of these sensitive lower layers. Below 2,500 A.U. the absorption in the dead horny layer increases rapidly and no radiation shorter than 2,400 A.U. reaches the living part of the epidermis as the absorption by the corneum is so complete as to prevent any of these waves from reaching the living layers of the skin. Live and dead tissue reacted the same as long as the dead tissue was kept moist with Ringer's solution. They use the term "passive absorption" to denote the maximum absorption at 2,750 A.U. in the corneum where this layer is not physiologically affected by the rays. "Active absorption" is the term they used to denote the increased absorption beginning at 3,130 A.U. caused by selective protein absorption producing biological reactions in ratio to the absorbed energy.

Thus, Tacher and his co-workers have conclusively demonstrated that the various layers of the skin exert a very different absorption and each layer has a characteristic absorption band. As mentioned previously, they also showed that ultraviolet penetrates deeper and stronger than indicated by Hasselbalch but not as strong and deep as indicated by Mecht and his co-workers.

The erythema and pigmentation spectrum

For that the primary reactions of the skin to ultraviolet light have been established as well as the degree of absorption of this light by the epidermis, the portion of this spectrum causing erythema and pigmentation can be discussed. Bruser and Valle (19[illegible], 19[illegible]7) gave the first action spectrum for the erythema and pigmentation of human skin. They found that vis-

ible light and long ultraviolet waves are ineffective in causing pigmentation and erythema. Erythema production begins in the ultraviolet region near 3,000 A.U., rises suddenly to a maximum at 2,970 A.U., then falls sharply to a minimum at 2,800 A.U., again rises to a second maximum at [illegible] at 2,500 A.U. after which it seems to fall although they were unable to determine this. Thus, they found that erythema occurs between 2,400 and 3,100 A.U. with two sharp maxima near 3,000 and 2,500 A.U. This happened regardless of the type of skin irradiated, whether it was negro or white. They also observed a qualitative difference in erythema, according to the exciting wave-lengths; the shorter the waves, the faster the rise and fall in time of erythema and pigmentation. Also, with short waves the erythema lasts only a short time and the pigmentation is slight. Erythema caused by the longer waves lasts long, disappears slowly, and leaves a strong pigmentation. Hausser (1928) investigated the transmission spectrum for the corneum and showed that this layer has a maximum of absorption at 2,800 A. U. (indicating an essentially protein character), which corresponds to the deep minimum of the erythema spectrum at that wave-length. Hausser was the first to point out that this maximum of absorption by the corneum accounts for the minimum in the erythema spectrum. He has also shown that the relative rate of development of erythema is different for different individuals due to some inherent cause. Schverin (1929) agrees with the results of Hausser and Vahle that maximal irradiation of erythema is at 2,970 and 2,500 A.U. The observations of Bachem (1929) and Bachem and Reed (1931) on the differential ab-

sorption of ultraviolet light by the various skin layers support the findings of Hausser and Vahle. These observations indicate that erythema production occurs in the germinal layer or the corium since the upper layers absorb very strongly at 2,800 A.U. and the stratum germinativum and the corium do not show an increased absorption at this wave-length but exhibit a gradual increase of absorption. This explains the decrease in sensitivity as was observed by Hausser and Vahle at 2,800 A.U.

Eachem and Kuntz (1929) attempted to explain the skin sensitivity curve of Hausser and Vahle on the basis of active and passive absorption in the epidermis. As has been previously explained they defined passive absorption as the maximum absorption at 2,800 A.U. occurring in a layer of dead tissue which is not physiologically affected by these rays, and active absorption as increased absorption beginning at 3,130 A.U., caused by selective protein absorption, producing biological reactions. In this case, the passive absorption of the rays at 2,800 A.U. could not cause erythema because the rays were all absorbed by dead cells which were unable to produce reactions of any sort; whereas the active absorption of all other ultraviolet rays could cause erythema, as these rays were absorbed by the living cells of the lower skin layers and these cells were capable of producing biological light reactions.

Uhlmann (1930) disagreed with Hausser and Vahle in their findings that both pigmentation and erythema occur at the same wave-lengths. Hausser and Vahle said that pigment production paralleled erythema production, that erythema is mainly form-

ed at 2,000 A.U. with a second maximum at 2,500 A.U. and that pigment formation occurs at these same spectral lines. However, Uhlmann claims that there is no parallel, even though he confirms Hausser and Vahle in that pigment is formed at the same time as the erythema. He points out that maximum pigment production is at 2,400 and 2,540 A.U. with a second small maximum at 2,970 and 3,003 A.U. This shows that the pigment formation occurs at the opposite end of the effective spectral range to the erythema formation. However, Luckiesh and Taylor (1939) are not in full accordance with the conclusion reached by Uhlmann that there is greater tanning at the longer ultraviolet wave-lengths and little tanning by the shorter rays. The findings of Luckiesh and Taylor indicate that ultraviolet energy in the wave-length region from 2,500 A.U. to 2,600 A.U. can cause a strong erythema, visible a few hours after exposure, but the inflammation subsides and disappears after a few days, leaving little or no tan. However, the energy of wave-lengths longer than approximately 3,300 A.U., especially that of 3,650 A.U., produces what appears to be direct tanning effect but with no erythema. The tanning, though, is very slight.

To obtain a good tan and one that would last for two months or more, Luckiesh and Taylor exposed twelve subjects to an artificial sunlamp at a distance of two and one-half feet. Twelve spots on each subject were exposed for times ranging from two and one-half to ten minutes with ultraviolet bands of 2,967 A.U., 3,022 A.U., and 3,130 A.U. Most of the erythema and tan were produced by lines at 2,967 and 3,022 A.U. These

results indicate that if tanning is desired, the exposures should be long enough and intense enough to produce some erythema. Maximum tanning is obtained at the same point as maximum erythema. Thus, the action spectrum for the pigmentation of the epidermis is the same or very similar to the erythema spectrum. Henschke and Schulze (1939a) agreed with these results arrived at by Luckiesh and Taylor.

Nevertheless, Luckiesh and Taylor (1939) agree with Uhlmann (1930) that the tanning spectrum has a considerably longer wave-length limit than the erythema spectrum. Furthermore, when applied in comparable dose, sunlight or carbon arc radiation, both of which are rich in wave-lengths longer than 3,200 A.U., produce a deeper tan than mercury arc radiation which is weak in such wave-lengths but strong in the shorter ultraviolet wave-lengths. However, the process by which this occurs will be reserved for later discussion.

Further investigations as to the action spectrum which is responsible for erythema were carried on by Coblentz, Stair, and Hogue (1931). They corroborated Hausser and Vahle's work by subjecting human skin to various ultraviolet wave-lengths. Their findings showed that erythemic response is nil below 3,130 A.U., increases rapidly with a decrease in wave-length, reaches a maximum around 2,970 A.U. and falls sharply to a minimum at around 2,800 A.U. At wave-lengths shorter than 2,800 A.U., the erythema response increases rapidly with a further decrease in wave-length; and a second maximum is reached at 2,500 A.U., with a lower value of sensitivity than data ob-

tained by other investigators. They claim that this disagreement of the spectral response curve at the region of 2,500 A.U. is due to the various methods used to describe the minimum perceptible erythema, which is very transitory for short wavelengths.

Luckiesh, Holladay, and Taylor (1930) also conducted experiments along these lines and they too obtained a maximum erythema around 2,970 A.U., followed by a sudden drop at 2,900 A.U., and then rising again to a second maximum at around 2,500 A.U. However, their second maximum was higher than found by previous investigators. Again, this difference may be attributed to the method used to describe the minimum perceptible erythema. The following figure gives a comparison of the results reached by Hausser and Vahle (1927), Luckiesh, Holladay, and Taylor (1930), and Coblentz, Stair and Hogue (1931).

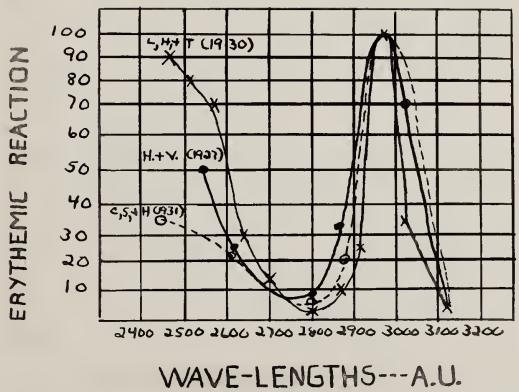

Figure 4. Action spectrum for erythema production. Coblentz, Stair and Hogue (1932).

Coblentz, Stair, and Hogue (1930) found that there is a reciprocal relation between the intensity of ultraviolet light and the time of exposure necessary to produce erythema. They noticed that the lower the intensity, the longer the exposure needed to cause erythema and vice versa. This being the case, they concluded that the erythrogenic power of ultraviolet radiation depends upon the intensity of the rays and upon the susceptibility of the skin to different wave-lengths. It appeared to them that the spectral erythemic response curve of the human skin is practically the same for different persons, in spite of the fact that the total energy required to produce an erythema is markedly different. Hausser (1928) also showed that the relative rate of development of erythema is different for different individuals even though the spectral response curve is alike for all.

The final figures as to the erythema spectrum, and the ones which hold today as the standard in this work, have been arrived at by Coblentz and Stair (1934). On further investigations in the erythemic spectrum, the authors have found it necessary to revise their figures on the wave-lengths which cause erythema. In a previous paper (1931), they claimed that the erythemal spectrum ranged from 2,400 A.U. up to 3,130 A.U.; but upon further experiments with more refined methods, they have had to extend the upper limits of the spectrum to 3,150 A.U. They claim that their error was due to the fact that in the spectral range where both erythema and pigmentation are produced, the longer the wave-length the greater appears to be the pigmentation rela-

tive to the erythemic reaction. Furthermore, since pigmentation begins before the erythema has disappeared in the case of the long wave-lengths, and since there is a similarity in color of the skin for these two reactions, there is difficulty in deciding when the erythema has disappeared. As a consequence, there is a possibility of over-estimating the magnitude of the erythemal reactions and, for this reason, the original figure setting the upper limit of the spectrum at 3,130 A.U. is lower than it rightly should be.

The site of the primary changes

Before proceeding further with a discussion of erythema and the parts of the spectrum causing erythema, it is first necessary to make note of the site of the primary changes caused by the erythema spectrum. Not much work has been done on this point as most of the investigations have been concerned with the histological changes which manifest themselves after the appearance of the erythema. Keller (1934) published the first paper on this subject. He found that the principal histological changes caused by sunburn radiation occur in the prickle cells where degeneration of the cells takes place. However, there were points of disagreement which he claimed were due largely to differences in the dosage of ultraviolet and in the time at which the biopsies were made; but the same general picture was present in all studies. The basal cell layer is not appreciably affected even at the tips of the papillae, which may be closer to the surface than some of the prickle cells. In no case have histological

changes been observed prior to erythema, at which time enlargement and engorgement of the capillaries become marked: intracellular edema, and the migration of leucocytes into the epidermis and into the corium in the region of the capillaries appeared about the same time or somewhat later. As early as twenty four hours after the exposure to ultraviolet radiation, degenerative changes in the prickle cell layer are detectable; these progress and eventually may involve all of this layer. The basal cells are less affected as a rule, but also may show degenerative changes; repair usually takes place through proliferation of cells in this layer. When the acute stage is passed, all layers of the epidermis, with the exception of the basal cell layer, but including the corneum, are usually left thickened.

Guillaume (1907) agrees with Keller that the principal histological change caused by sunburn radiation is a degeneration of the prickle cells, although this does not appear until some time after the irradiation, and long after erythema has appeared. Keller (1911), after further investigations on this subject, confirms his paper of 1904 to the effect that there are numerous physical changes in the skin after irradiation. Miescher (1930) found that with large doses, endothelial cells and fibroblasts of the corium, as well as epidermal elements, show degeneration. The latest investigation on this subject by Marperl, Henschke, and Schulze (1939) points out that the nuclei of the most superficial cells of the epidermis are the first to show degeneration, and they suggest that this occurs because these cells receive more ultraviolet radiation than those more

deeply placed.

Thus, it seems probable that the principal photochemical changes leading to sunburn must be most intense in the epidermis since sunburn radiation penetrates very little below this layer. However, the investigators are at variance as to whether these primary changes occur before the appearance of the erythema or after its appearance. Nevertheless, the principal site of the initial photochemical changes seem to be in the prickle cell layer, although there is a certain degree of accumulation of blood cells in the papillary layer and a degeneration of nuclei of the cells in the more superficial layers. These latter changes are probably secondary. Both the malpighian layer and the corneum subsequently become thickened.

The latent period

Between the time of the initial radiation of the ultraviolet light and the first appearance of a just perceptible erythema, there is a long latent period which indicates a certain degree of separation between the primary photochemical process caused by the irradiations and the final result. This separation is further attested by the effects of temperature on the erythemic process which have been studied by Clark (1935,1936). She finds that the threshold time, i.e., the period of irradiation required to produce a just perceptible erythema, is very little influenced by temperature; but that, on the other hand, the latent period for the appearance of erythema is markedly affected by temperature, having a temperature coefficient of 1.5. Clark claims that a difference in the temperature coefficient of these

two processes indicates that the erythema mechanism is composed of two rather distinct parts. The first part is a photochemical reaction which determines the amount of radiation necessary to produce the erythemal response. This process, like most photochemical reactions, has a temperature coefficient of one. This reaction takes place principally in the prickle cell layer, but sets off other reactions which result in the production of substances which cause the dilation of the minute vessels in the papillary layer. It is these secondary reactions which occupy the latent period, and which are dominated by a thermal process so that the overall temperature coefficient is relatively high. The full implications of Clark's findings, aside from establishing the latent period as two distinct processes, will be discussed later.

The absorption substance which initiates erythema

As has been said, a close resemblance between the action spectrum and absorption spectrum is to be expected if only a relatively small fraction of the incident light is absorbed. In the case of sunburn, however, close resemblance could hardly be expected since the epidermis absorbs such a large portion of the incident radiation. It is therefore somewhat difficult to arrive at the absorption spectrum of the absorbing substance which initiates sunburn from a consideration of the action spectrum for erythema production. Blum (1941) has pointed out that if the photochemical processes occur only in the prickle cell layer, as demonstrated by Fellner (1941, 1941) and Guillaume

(1947), the cornea and granulomatous tissue form a slight filter which absorbs short wave-lengths to a greater extent than others. Thus, the radiation which reaches the prickle cell layer is quite different in spectral distribution from that incident at the surface of the skin. The prickle cell layer itself is relatively thick and should modify the action spectrum of a photochemical reaction taking place in it. Consequently, the true determination of the absorbing substance which initiates the erythema is made increasingly difficult by all of these variables involved and any conclusions reached concerning this absorbing substance must be tempered with caution.

In a previous section of this paper, the results of Hanri (1912) and Cosparson (1936) were presented to demonstrate that both proteins and nucleic acids have characteristic absorption spectra in the region of the ultraviolet. Subsequently, further investigations have been undertaken to determine which of these substances initiates erythema. As a result of these additional studies, many investigators have found that both nucleic acids and proteins can cause photochemical reactions to occur. Turner (1938) found that a transmission spectrum for the corneum shows that this layer has a maximum absorption at 2,800 A.U., which indicates its essentially protein character since proteins show maximum absorption at around 2,800 A.U., whereas nucleic acids absorb maximally around 2,600 A.U. He correlated his results with the fact that the deep minimum of the erythema spectrum also occurs at this wave-length. Rajewsky (1938) gives a method for studying in vitro radiation effects similar to those in

live animal tissues. This is done by irradiating an albumin preparation with ultraviolet of varying wave-lengths and counting the number of coagulated particles produced, using an ultramicroscope for this purpose. The number of such particles varies with the wave-length of the radiation, other factors remaining the same. This variation parallels that in the production of the skin erythema by the same rays. Thus, he shows a comparison of erythema reaction with reaction of irradiated proteins and shows the dependence of each on the same wave-length of light. Lucas (1931) has also demonstrated that the shape of the absorption curve of the epidermis resembles those of many proteins and amino acids, such as serum-albumin, caseinogen, tryptophan, and tyrosine. Mitchell (1934) has attempted to explain the reason for the large differences in the maxima of the action and absorption spectra that cause erythema by assuming that the corneum acts as a filter and that the proteins of the Malpighian layer are the light absorbers. He estimates the latter as equivalent in absorbing power to 0.005 cm. of a 6% solution of a "typical protein" (ovalbumin is chosen as such). He arrives at the conclusion that proteins are the light absorbers for the erythemic mechanism, and that the displacement of the maximum of the action spectrum (about 2,970 A.U.) from the maximum absorption of protein (about 2,800 A.U.) can be adequately accounted for by the strong absorption of the corneum at short wave-lengths with a maximum at 2,800 A.U., which must shift the maximum of the action spectrum to longer wave-lengths. Finally, Rothman and Rubin (1940) suggest that para-amino-ben-

zoic acid is the light absorber, but they have not made a thorough analysis of the agreement of the absorption spectrum of this substance with the erythema spectrum. Nevertheless, they found that when solutions of this compound, which had been exposed to ultraviolet radiation, were injected intradermally, erythema of the local area developed after an appropriate latent period. These changes did not occur in the absence of molecular oxygen; whereas Blum, Matrous and West (1935) have shown that deprivation of oxygen during the period of exposure to ultraviolet light does not inhibit the subsequent development of erythema.

However, numerous other investigations have demonstrated that nucleic acids are perfectly capable of initiating photochemical responses to ultraviolet irradiations. For this reason, the factor of nucleic acids cannot be entirely ruled out as having some consequence in the subsequent development of erythema. But many of these studies have been conducted on media other than the skin; and, thus, care must be exercised in trying to relate these results to reactions occurring in the epidermis. Giese (1938) found that following irradiation of fertilized eggs of the sea urchin Strongylocentrotus purpuratus with known doses of monochromatic ultraviolet, no acceleration of cleavage was observed; but retardation was obvious when the dosage was large enough. 2,804 A.U. was most effective in retarding while wave-lengths at 3,660 A.U., even at the highest dosages, produced barely perceptible effects. This lack of correlation of the wave-length of maximum extinction of light by the eggs

with the wave-length of maximal efficiency might be taken as an indication that the destructive effect is mediated by some substance especially sensitive to 2,804 A.U., namely proteins. Yet, Giese (1939b) found that the sperm of this same sea urchin are readily affected by ultraviolet radiations of 2,654 A.U. and 2,804 A.U., the first more so than the second. Radiations of 2,654 A.U. are more completely extinguished than those of 2,804 A.U. Since the sperm is practically a naked nucleus, the data suggests that the primary effect of ultraviolet radiations upon these cells is an effect on the nucleus and, thus, the nucleic acids therein. Upon further investigation, Giese (1939a) noted that Paramecium caudatum grown under controlled conditions shows a relatively constant division rate; but after irradiation with ultraviolet light, the division rate is decreased, the retardation being proportional to the dosage. The retardation produced by a given dosage with 2,804 A.U. is greater than the retardation produced by a similar dosage at 2,654 A.U. However, recovery of Paramecia from injury produced by 2,804 A.U. is more rapid than recovery from injury by 2,654 A.U. This suggests localization of the destructive effects of 2,804 A.U. in the cytoplasm, the effects of 2,654 A.U. in the nucleus. Giese (1939c) discovered that the eggs of Urechis caupo irradiated with ultraviolet light showed retarded and irregular cleavage. Wave-lengths at 2,804 A.U. were found to be more effective in retarding cleavage than wave-lengths at 2,654 A.U. Eggs extinguish the irradiations more strongly at 2,804 A.U. than at 2,654 A.U.; for the sperm the reverse is the case. Thus, these

observations of Giese suggest that either protein or nucleic acid, and either the nucleus or cytoplasm may be affected, depending upon the nature of the system and the experimental conditions. Unfortunately, the general similarity of these absorption spectra, and limitations imposed on the measurement of action spectra by the nature of the systems studied, e.g., screening action of the superficial layers, render comparisons uncertain. Hence, conclusions drawn from this type of measurement must always be viewed with care.

Further studies along this line of investigation have favored nucleic acids more predominately as the absorbing substance for causing photochemical reactions. To illustrate this, Landon (1939) observed the results on spores and sporidia of Ustilago zeae when treated with monochromatic ultraviolet light. After incubation, they were observed microscopically to determine the fraction surviving the irradiations. Using 50% killing as a criterion, there is a maximum sensitivity of both spores and sporidia at about 2,650 A.U., which indicates nucleic acid as the absorbing substance. Landon and Uber (1939) did absorption measurements on eight micra thick layers of two day and sixteen day old yeast cell (Saccharomyces ellipsoideus). The measurements on the yeast cells, pressed between quartz plates, were made at fifteen wave-lengths between 3,650 and 2,300 A.U., using mercury emission lines. An absorption maximum at 2,650 A.U. is found for the active cells; this maximum decreases in the case of the sixteen day old cells, and reveals a secondary maximum at 2,800 A.U. Thus, at one point there is nucleic acid absorp-

tion and, at a later time, absorption by proteins. Hollaender, Jones, and Jacobs (1940) studied the effects on Entrophius variicularis eggs irradiated with measured quantities of monochromatic ultraviolet between 2,880 and 3,150 A.U. Survival was evaluated from the percentage of the eggs hatched. Wave-length dependence on inactivation of the eggs shows a small maximum at 2,805 A.U. and increased sensitivity below 2,600 A.U. This demonstrates nucleic acid to be the main absorbing substance.

These findings have been presented to illustrate the point that nucleic acids as well as proteins are capable of initiating biologic reactions in response to irradiations by ultraviolet light. However, as has been mentioned previously, the media used did not include the skin and, hence, it is difficult to interpret these results in relation to reactions that occur in irradiated skin. It was not until Clark (1935,1936) gave her conclusions on the temperature coefficients of proteins and photochemical reactions causing erythema that anything definite could be stated in regard to proteins not being the absorbing substance which causes erythema. Clark's observations on this subject were very extensive and merit considerable attention as to the true nature of the absorbing substance. She found that the coagulation of proteins (she used an isoelectric egg albumin solution) by ultraviolet radiation involves three distinct processes. The first is the light denaturation of the protein molecule which has a temperature coefficient of one. The second is a reaction between the light denatured molecule and water which has a temperature coefficient of eight to ten.

The third, and final process, is the flocculation of light and heat denatured molecules to form a coagulum. Clark noted that the initial change produced in skin tissue by ultraviolet radiation had a temperature coefficient of about one but that the temperature coefficient of erythema production was 2.3. A similarity between the initial photochemical reaction in irradiated skin and the coagulation of proteins is suggested by the fact that the initial photochemical change in the skin has a temperature coefficient of about one and the initial light denaturation change in the proteins also is one. A further similarity between the two processes lies in the fact that both protein coagulation and erythema production show a definite latent period. However, at this point all similarities between these two processes cease to exist. Additional analysis of the temperature coefficients of the two reactions demonstrate that although both have a latent period, the latent period of the erythema production has a temperature coefficient of 2.3, whereas the latent period of protein coagulation has a temperature coefficient of eight to ten. Thus, Clark concludes that the production of erythema which is supposed to be due to the release of a vasodilator substance in damaged tissue, is probably not related to coagulation of tissue proteins by ultraviolet radiation, since the temperature coefficient of the latent period in erythema production would be of the order of magnitude of eight to ten instead of 2.3.

Finally, Hamperl, Henschke, and Schulze (1939) have presented observations which favor nucleic acids as being the light

absorbers which initiate erythema. Their findings are in answer to Mitchell (1938) who accounts for the erythema spectrum in terms of proteins. These authors state that they have found maxima in the erythema spectrum at [illegible],950 A.U. and [illegible],600 A.U. with a minimum at [illegible],750 A.U., and, for some skins with a very thin corneum, another maximum at [illegible],300 A.U. These figures resemble more closely the absorption spectrum of nucleic acids than the absorption spectrum of proteins. In additional support of their thesis, these investigators also point out that the nuclei of the most superficial cells of the epidermis are the first to show degeneration; and they suggest this occurs because these cells receive more ultraviolet radiation than those more deeply placed.

In the light of all the evidence presented it is extremely difficult to draw any valid conclusions as to the true nature of the absorbing substance which initiates erythema. Since both proteins and nucleic acids have absorption spectra in the same range as the erythemic action spectrum, and since both are important constituents of all cells in the skin, it may be possible that the production of erythema is a result of some complicated interaction between proteins and nucleic acids. Until additional investigations are carried on to prove conclusively which substance is the cause of erythema or the process by which they interact, all that can be assumed is that erythema is a sequel to injury to the prickle cells produced by wave-lengths shorter than 3,300 A.U.

Theories of the mechanism causing erythema

It is generally agreed that the initial photochemical reaction brings about the elaboration, in the epidermis, of a substance which migrates to the region of the minute vessels in the papillary layer and brings about their dilation. Since the sunburn wave-lengths do not penetrate to the papillary layer to any appreciable extent, there can be little or no direct action on these vessels, and the long latent period between exposure to irradiation and appearance of erythema also indicates an indirect effect. The nature and mode of elaboration of this dilator substance has been the subject of a number of hypotheses.

While conducting experiments on the vascular reactions of the skin to ultraviolet light, Lewis and Zotterman (1936) observed that the reaction of cutaneous vessels to ultraviolet light consists essentially of three parts: a local and active vasodilation; a reflex dilation of the muscular arterioles; and, locally, increased permeability of the vessel walls. The vasodilator substances produced by ultraviolet radiation of the skin diffuse into the surrounding skin and are carried away by the lymphatic channels. Lewis (1937) has attempted to relate this vasodilator substance to the subsequent appearance of erythema. He has presented considerable evidence that erythema always results from the local elaboration of a histamine-like substance which he calls the "H" substance to show its similarity to histamine and at the same time to avoid too definite a statement as to its true chemical nature. If hista-

mine is pricked into the skin, there then follows a reaction known as the "triple response" of Lewis. Erythema immediately appears in a small area surrounding the prick; this is followed shortly by edema of the same area, and finally, a "flare" of erythema which spreads outward from the edematous region. The same sequence of events is seen in spontaneous uticaria ("hives"), in dermatographia (abnormal response to mechanical stimulation), and in certain other responses of the skin. Lewis believes such phenomena to result from the action of a histamine-like "H" substance; and he also explains the erythema of sunburn in the same way, assuming that the dilation of the minute vessels results from the production of an "H" substance in the skin.

Krogh (1929), however, believes it is unnecessary to assume more than one dilator substance to explain all the different types of erythema, and in the case of sunburn thinks that it cannot be the same as Lewis' "H" substance. The character of erythema of sunburn certainly differs from that of uticaria, in that edema is not a common sequel but only follows severe dosages, and in that the latent period is much longer than that which lapses between the introduction of histamine into the skin and the appearance of erythema, or between mechanical stimulus and response in dermatographism. The long latent period of sunburn might represent either the time required for the elaboration of the dilator substance or for its penetration to the vascular layer of the skin; and the non-appearance of edema might be due to the fact that because of its slow elaboration, the dilator substance never reaches a very high concentration before it is

removed. Krogh concludes that in any case it seems unwise either to accept or reject the "H" substance hypothesis for the erythema of sunburn without further evidence.

Ellinger (1926) found that a histamine-like dilator substance is present in greater quantity in irradiated than in normal skin. In 1929 and 1930 he proposed that the erythema of sunburn results from the direct production of histamine from histidine by a photochemical reaction. He studied the absorption spectrum of a histidine preparation in the region of the erythemic spectrum and found that the exposure to a mercury arc resulted in the formation of a substance having the biological properties of histamine. This seemed good evidence that the light absorbing molecule which initiates the erythemic response is histidine, and that the mechanism is a simple photochemical production of histamine from this substance in the skin. However, Bourdillon, Codd, and Jenkins (1930) were unable to confirm Ellinger's measurements of the absorption spectrum of histidine, finding no appreciable absorption of wave-lengths longer than 2,550 A.U.; but that wave-lengths shorter than this are far more active in the formation of histamine from histidine than longer wave-lengths. These facts do not support the suggestion that the erythema following irradiation of the skin is due to the formation of histamine from histidine by the simple chemical process which may be demonstrated in vitro. Furthermore, these workers held that the production of the active compound by the physiologically active ultraviolet wave-lengths was too slow to account for the production of ery-

tions. Ellinger (1933c) repeated his experiments on purified histidine and confirmed the findings of these investigators. However, he was able to separate from his original histidine preparation an iron-containing substance whose absorption spectrum corresponded quite well with that of the hemochromogen spectrum. He suggested that this impurity could be a determinant in the production of histamine.

These views on the irradiation of cow's liver which is supposed to lead to the formation of a histamine-like substance, as claimed by Ellinger and Lewis, were opposed by Saendre (1931). Since no chemical evidence had yet been adduced as to the possibility of a photochemical change of histidine to histamine itself, Saendre irradiated solutions of pure histidine with a mercury vapor lamp and then treated the product chemically for the separation of histamine and tested the histamine fraction physiologically (stimulant action upon isolated intestine of a guinea pig) and chemically. The histamine fraction acted like histamine but it gave no precipitate with picric, picrolonic, or rufianic acids as histamine did; chloride of gold or platinum or silver nitrate formed no addition product as with histamine, but reduction occurred. In the irradiation more ammonia was given off than CO_2. These results pointed to the formation of imidazol-acetaldehyde by irradiation; this was confirmed by separation of the phenylhydrazone. Saendre concludes that the physiologically active substance formed by irradiation of solutions or tissues containing histidine is, therefore, not histamine but imidazol-acetaldehyde. However,

this reaction requires the presence of oxygen; and Dodge (1936) finds that the substance of histamine-like nature formed by the irradiation of histidine with a quartz mercury vapor lamp is quantitatively greater in yield when irradiated at alkaline reaction and in nitrogen than at neutral or acid reaction and in oxygen. Frankenburger (1933), who also entertained the hypothesis that histamine was formed by light, and Blum, Weitrous, and West (1935) also find the dilator substance to be formed in the absence of oxygen. Moreover, erythema production, like other biological effects of ultraviolet radiation, proceeds in the absence of oxygen. This idea of greater histamine formation in the presence of alkali was held also by Lyalkova and Solowaitschik (1937) who found that, following an irradiation of three minutes at one centimeter, a reddening of a rabbit skin appears within three to twenty-four hours, and the pH rises parallel with the irradiation. Hyslanely (1938) reported on experiments on rabbits in which two large portions on the back were shaved and then exposed to ultraviolet rays, after which the skin was removed, dried, and a chemical analysis made. He found that the cations had decreased and the anions increased or unchanged so that the medium of the skin in which the photochemical processes were occurring was decidedly on the alkaline side. Proft (1931) observed that the increase of the light erythema is due neither to the presence of pigment nor to the swelling of the corneus epithelium; but it is closely linked to the presence of an alkali, larger concentrations being less effective. The formation of erythema is antagonized by oils,

fats, and undissolved soaps; and a 10% alcoholic solution of salicylic acid gave absolute protection. It is suggested that the increase of the light erythema is due in part to the formation of alkali proteinates under the influence of ultraviolet light, and that these or their decomposition products are the chief causes of the increase in erythema.

These latter investigations have been presented to demonstrate that the primary photochemical processes which ultimately result in erythema and pigmentation, whether it be from the formation of histamine or some other breakdown product, occur in the absence of any oxygen and in a medium that is decidedly alkaline.

Further investigations have been made which appear to disprove the histamine hypothesis. Grant and Wood (1938) pricked histamine into human skin so that a wheal developed. The wheal was excised and compared microscopically with skin that had no wheal. It was found that histamine had no appreciable power to cause emigration of leucocytes from the blood vessels when injected intraperitoneally or applied to the exposed mesentery or the conjunctiva in the frog, when instilled into the conjunctival sac of the rabbit or when pricked into the human skin. Thus, they concluded that release of histamine doesn't provide a full explanation of the process of inflammation. Abramson (1940) found that ultraviolet light could cause a wheal that could not flare at the sides, so the pattern of the wheal matches the pattern of the zone irradiated. The absence of a flare is not in accord with the theory that a readily diffusable "H" sub-

stance like histamine is liberated in the tissues subsequent to irradiation, since, according to Lewis (1927), release of histamine is supposed to cause a flare to form. An irradiated zone of the skin which had many needle punctures, so as to damage the cells, was covered with wet cotton and a negative pole to electrically transfer histamine from the skin during the wheeling process by reverse electrophoresis of histamine. However, no wheeling substance resembling histamine was isolated from the skin. Abramson concluded that these observations do not support the point of view that histamine or a readily diffusable "H" substance is responsible for the skin response to ultraviolet radiation. Recently, Menkin (1943, 1944) isolated from inflammatory exudates several substances which act specifically to bring about certain of these tissue responses; and it seems probable, according to Menkin, that the complicated picture which inflammation presents may ultimately be explained in terms of such "inflammation-substances". He has brought forth evidence that the increase in capillary permeability which occurs in inflammation is not due to histamine, as supposed by Lewis and others, but to a substance which he calls "leucotaxine". This substance brings about the migration of leucocytes from the capillaries into the surrounding tissue as a result of cell injury, such as is caused by ultraviolet irradiation. Menkin believes that tissue edema, as seen in erythema of sunburn, is a direct manifestation of increased capillary permeability caused by leucotaxine and not by histamine.

The influence of nerve innervation on the production of

erythema has been described by Rothe (1927). He observed that when the nervus auricularis minor and the nervus auricularis magnus of a rabbit were cut on one side, both of the ears as well as the skin of the head having been depilated with sodium sulfide, the production of erythema following irradiation was decreased. This was due apparently to the loss of some process dependent on the intact nerve supply. These results of Rothe have received support from irradiation in the guinea pig by Clunet, Cardot, and Hofman (1928), after cutting the nervus auricularis magnus at the base of the ear. Irradiations of five, ten, and thirty minutes on the normal ear caused perfectly localized erythema with intensity varying according to the amount of exposure, followed by a slight pigmentation. On the operated ear the five and ten minute irradiations caused no effect while the thirty minute irradiation produced a slight redness, appearing after about twelve hours and disappearing at the end of the second day. This shows that the formation of erythema and pigmentation is largely dependent on an intact nerve supply. They also anesthetized one side of the pig and subjected them to irradiations. Irradiations of the normal side for two to five minutes produced a slight redness, appearing in about twenty-four hours and disappearing on the second or third day, but no reaction on the anesthetized side. Irradiations of fifteen to thirty minutes caused a durable pigmentation and thickened epidermis on the normal side; on the anesthetized side, a slight redness appeared after not less than thirty-six hours and disappeared on the third day without leaving traces. This experiment

also demonstrates the importance of a proper functioning nerve supply in reactions to ultraviolet irradiations.

The phenomenon that may influence the erythema threshold of an individual to ultraviolet radiation was described by Blum and Terus (1940a). They first noted that erythema develops best at an optimum dosage which varies for people. The authors then found that the development of erythema may be inhibited, partially, by the application of heavy doses of long ultraviolet wave-lengths within the sunburn-producing spectrum, but not with short ultraviolet or visible light. They suggest that this is due to the fact that higher doses partially inhibit the minute superficial blood vessels directly, whereas the erythema itself depends chiefly on the release of dilator substances by the injured cells of the epidermis. This may also serve to explain the latent period.

Several serious objections have been put forth by Blum (1941) concerning the hypothesis on the formation of erythema by the elaboration of a histamine-like substance. He first points out that the long latent period which intervenes between the action of radiation and the appearance of erythema would demand that the histidine molecule retain its activation from the irradiations for periods as long as several hours before breaking up into histamine and carbon dioxide. Regarded from a photochemical point of view, this is an extremely remote possibility. The reaction proposed is a direct one with no intermediate steps which might be delayed in one manner or another. However, Blum proposed that this difficulty might be overcome if it could be

assumed that the histamine is immediately formed on the action of light on histidine in the epidermal cell layer, but if a long latent period is needed for the diffusion of the histamine to the capillary layer where it can effect dilation of the erythrocyte blood vessels. Nevertheless, he admits that there are certain objections to this argument. Both Ellinger (1930) and Freudenberg (1933) found it necessary to irradiate histidine solutions for long periods with intense quartz mercury arc radiation before any detectable amounts of histamine could be obtained; but the formation of histamine in vivo would have to occur in very short periods of time since only a few minutes, or even seconds, are required to produce erythema in the intensity of the sunburn radiation in bird species. The distance for diffusion is very short, so that the rate of diffusion cannot be very slow to provide the long latent period which occurs under normal conditions. But the temperature coefficient found by Clark (1936) for the latent period could hardly be characteristic of a diffusion process, which should have a temperature coefficient near one instead of 2.3. Because of the latter objection, Blum concludes that it is difficult to account for the erythema of sunburn by any hypothesis which regards the formation of a dilator substance by a direct photochemical process.

Mitchell (1938) postulates that the "RW substance" is a high molecular weight breakdown product of protein, which diffuses very slowly. This explanation is more probable, according to Blum (1941), than that of direct histamine formation; but it still leaves the high temperature coefficient of the latent period un-

explained. Elman at the start of elaboration to such hypotheses may be formulated upon the already determined fact that injured cells are known to release some kind of dilator substance. If the action of ultraviolet light is assumed to injure cells in the epidermis, dilation of the blood vessels should result due to the release of such a dilator substance by the injured cells. The latent period should then represent the time required for the liberation of the dilator substance from the injured cells; and the process might well have a temperature coefficient similar to that found by Clark (1936). Such an hypothesis will also account for the development of pigmentation, which is a common response of epidermal cells to injury regardless of the injurious agent. The introduction of histamine into the skin results in the triple response, but does not result in pigmentation; whereas injury to the epidermal cells may be followed by a similar triple response and then by pigmentation.

Clark (1935, 1936) proposed that sunburn results from the coagulation and denaturation of the skin proteins by ultraviolet light; but often can fully studying the temperature coefficients of such processes and comparing them with those for the threshold and latent period of sunburn erythema, and finding no agreement, she abandoned the hypothesis. However, Blum (1941) points out that the rate of coagulation may be less important than the total amount of protein altered, which should depend on the quantity of light absorbed. The amount of protein altered should determine the extent of cell injury and, hence, the threshold of erythema, which Clark found to have a low temperature-

ture coefficient. The latent period, on the other hand, would be dominated by the rate of production of dilator substances by the cells, according to the foregoing hypothesis, and might be expected to have a temperature coefficient of 2.3 as found by Clark. Thus, Blum concludes that there seems to be no irrefutable objection to the hypothesis that the photochemical basis for sunburn is coagulation or denaturation of the protein in the prickle cells of the epidermis.

Despite the excellent arguements presented by Blum in support of his above hypotheses, it must be remembered that they have not been demonstrated in the laboratory. Although the earlier investigators on this subject arrived at their conclusions by actual experimentation, it is not necessary for the reader to accept their findings as conclusive. Blum's objections to their results are certainly logical and do bear much weight in view of his own studies. However, any decisions reached on this subject must be weighed carefully, as nothing conclusive has been proven by anyone as to the true nature of the dilator substance which causes erythema. Much research still remains to be done on this point. I believe that, at present, in view of the evidence given, it is impossible to reach any valid conclusions on what the dilator substance is, and all that is definitely known is that the elaboration of some substance by some means is ultimately responsible for the appearance of erythema.

Pigmentation

Pigmentation is usually observed much later than erythema;

[illegible] a rule the redder color changes almost imperceptibly to brown. Sometimes the erythema may be imperceptible, while pigmentation occurring [illegible] days with little or no earlier indication of sunburn. Since the degree and rate of pigmentation varies with the dosage of radiation, considerable differences in the histological picture may be expected. Miller (19[illegible]) finds that the first event in pigmentation is not the formation of new pigment "in situ", but the migration of pigment granules already formed in the basal cells to more superficial layers, including the corneum. It is found in bits of desquamated corneum which are cast off following a mild degree of sunburn. The actual production of new pigment does not commence until a considerable time after irradiation.

It seems certain that the primary changes which lead to pigmentation are the same as those which cause erythema, and that the former is a normal response to the latter. The action spectrum for the underlying photochemical changes is probably identical for the two processes, although there may be apparent deviations in the observed action spectra. Secondarily, a reaction which causes darkening of the skin pigment already present has been described. Although this seems to be of secondary importance, it has probably led to confusion in earlier attempts to compare the action spectra for erythema and pigmentation.

The pigment which follows exposure to sunburn radiation consists of granules of melanin. Block (1918) found that when histological sections are treated with the phenolic compound levorotatory 3-4 dihydroxyphenylalanine (l-dopa), melanin is

formed in the melanoblasts, presumably due to the presence of a substrate specific enzyme, dopaoxidase, in these cells. The dopa is brought to the melanoblasts by way of the blood stream and there it is converted to melanin by oxidation and polymerization through the action of the enzyme. Agreeing with Bloch, Peck (1929, 1930, 1931) determined that the most important factor for the production of pigment is light although injection of pyrrol bodies, acetic acid, dopa, and the mechanical trauma can cause pigmentation. Animals under the same experimental conditions but kept in darkness did not form pigment. The greatest amount of pigmentation was produced by the ultraviolet lamp, less by diffuse light, and least of all in the presence of light coming through the room windows. It seems, therefore, that ultraviolet light is especially strong in pigmentogenic properties. Peck noticed that the first evident step in the process of pigmentation was that the dopa reaction became strongly positive, before there was an increase in pigmentation, in the melanoblasts of the skin and that its duration and intensity were an index of the degree of pigment formation. From the beginning of pigmentation many dendritic cells not normally present are found in the epidermis between the basal cells and these dendritic melanoblasts become much more numerous than the nondendritic ones; at the height of pigment formation nearly all the melanoblasts showed a dendritic form. Thus, with an increase in the dopa reaction, i.e., in pigment-building activity, there was a concomitant increase in the number of dendritic cells; and with its decrease there was a decrease in the numberof dendritic cells. The

greatest number of dendrites was not seen at the time of the greatest pigmentation, but at the time of the greatest pigment-building activity. When the section showed its greatest pigment content, pigment production was already on the wane and with it the number of dendritic cells. The best and clearest dendritic cell pictures were also seen with the dopa reaction. The pigment, which was formed in the basal cell layer, is transported upward by the upward growth of cells and concentrated in the stratum corneum where it is usually removed by being cast off with the scales since very little is carried away by the chromatophores of the cutis. Peck claims that the only function of the dendritic cells that is at present recognized is their melanoblastic function. But basal cells of nondendritic form are also capable of building pigment, as they, as well as the dendritic cells, give a positive dopa reaction, i.e., contain pigment-building oxydase. Peck concludes that the most probable theory of the origin of the dendritic cells is that they are special functional phases of the nondendritic basal cells. As yet, this theory has not been proven.

The hypothesis that melanin is the skin pigment was further advanced by Lignac (1929) by protecting pieces of skin from ultraviolet radiations except for a cross-shaped portion which was fully exposed. Skin fixed in alcohol and formalin was used. After an exposure of three hours the skin became dark brown. Microchemical tests were then made and these showed melanin to be present in great quantities.

However, the idea that melanin is formed by the oxidation

and polymerization of the phenolic compound amino-acid dihydroxyphenylalanine (dopa) by the enzyme dopaoxidase is not at all in agreement with the findings of many other investigators. Verne (1930) believed it is more probable that otherphenolic compounds, particularly tyrosinase or some other oxidizing enzyme may promote this reaction. Both Verne (1930) and Lucas (1931) arrived at this conclusion when they found that tyrosine exhibited a characteristic absorption curve within the ultraviolet region that causes erythema whereas dihydroxyphenylalanine did not show such a curve. It must also be remembered that dopa is an amino-acid, and the findings of Kaplansky and Soloweitschik (1927), Kaplansky (1928) and Proft (1931), which were presented in a previous portion of thispaper, clearly demonstrate that the production of erythema and pigmentation occur in an alkaline medium. Tyrosine is an alkali.

Frankenburger (1933) suggests that pigment is formed directly by the action of ultraviolet radiation on tyrosin in the skin. He also suggested, as did Ellinger (1930), that histamine is formed by the reaction of ultraviolet radiation on histidine. Frankenburger proposed that the sunburn spectrum is made up of two parts, erythema production corresponding to the absorption spectrum of histidine, and pigmentation corresponding to the absorption spectrum of alkaline tyrosine. He found that histamine or histamine-like substance could be formed from histidine and that a brown substance was formed by long irradiation of tyrosin. The former reaction took place in an atmosphere of nitrogen, but the latter occurred only in the presence of oxygen. It may be

pointed out that an analysis which indicates similarity between the action spectrum of sunburn and the absorption spectrum of tyrosine must also show similarity between the absorption spectrum, since tyrosine and protein absorption spectra are very similar (Lucas, 1931). However, the same arguement may be brought against Frankenburger's hypothesis, for the formation of pigment by the direct action of ultraviolet radiation on tyrosin, which has already been advanced against Ellinger's proposal that histamine is formed from histidine in like manner. Both hypotheses demand that a molecule, either tyrosine or histidine is activated by the radiation, and remains in some sort of activated state during the long latent period which elapses before erythema or pigmentation occurs. Blum (1941) claims that this is incompatible with the photochemical theory.

This difficulty is avoided by the hypothesis of Arnow (1937) who follows the contention of Bloch (1926) that pigment is formed only by the direct action of dopaoxidase on dopa. He suggests that skin pigmentation produced by radiant energy is the direct result of the conversion of tyrosine to dopa, the latter being converted to melanin by dopaoxidase; but the dopa can be produced by this method only in the presence of oxygen. Thus the ultraviolet radiation can replace the enzyme tyrosinase in this first step of the reaction to bring about the oxidation of tyrosine to dopa. Arnow suggests that it is the first step of this reaction, the conversion of tyrosine to dopa, which might occupy the latent period.

Nevertheless, two serious difficulties confront Arnow's

hypothesis. In the first place Arnow's reaction, as did that of Frankenburger's, requires the presence of molecular oxygen, since they could get the reaction to occur only in vitro, for the formation of pigment. That pigment can be formed when the oxygen tension in the skin is considerably reduced during the time ofirradiation was demonstrated by Blum, Watrous, and West (1935). They found that depriving the skin of the forearm ofoxygen by means of a sphygmomanometer cuff had no apparent effect on the subsequent formation of the erythema and pigmentation of sunburn. The second serious objection to Arnow's hypothesis was that the exposures to ultraviolet radiation, which were required to convert the tyrosine to dopa, were much greater than those needed to produce suntan in human skin.

These objections to Arnow's hypothesis were the subject of a very extensive research by Rothman (1940) that provided far-reaching results in clearing up this question. He believed that 3-4 dihydroxyphenylalanine (dopa) is the immediate precursor to melanin which becomes oxidized to melanin by an intracellular specific oxidase present only in normal functioning melanoblasts. The problem is where does the dopa originate. Arnow (1937) demonstrated the formation of dopa by exposure of tyrosine solutions to ultraviolet radiations; but thisprocess does not show pigmentation until eight to thirty times longer than it takes in human skin. However, Rothman found that this process can be accelerated by the addition of ferrous salts so that the reaction now serves as a model of dopa formation in human skin. Mixtures of tyrosine and ferrous salts irradiated with threshold

erythema doses give much dopa but no melanin. Yet when these samples are kept in the dark, progressively increasing amounts of melanin are formed after sixteen to twenty-four hours and he suggests that this is the way the latent period of pigment formation in the skin is simulated. The dopa formation increases to a certain maximum and then remains unchanged because dopa formation and the oxidation of dopa to melanin, by the oxidase in the melanoblasts, keep balance with each other and melanin does not exceed a certain maximum as it is decomposed into lighter colored soluble products by the continued irradiation. Thus, ultraviolet radiation acts on tyrosine-ferrous salts similarly to tyrosinase and suggests that even in mammals which lack tyrosinase, tyrosine is the primary precursor of melanin and dopa formation may occur by ultraviolet rays even if non-specific oxidation catalysts arepresent.

These conclusions now validate the results of Evans and Raper (1937) who also, like Arnow (1937), found that the first step in the formation of melanin is the oxidation of tyrosine to dopa. They noticed that this process takes place very slowly and requires the participation of a catalyst, the enzyme tyrosinase. This finding opposes that of Blum, Watrous, and West (1935) who have shown that oxygen is not needed for pigment formation whereas the reaction of Evans and Raper, when done in vitro, requires the uptake of oxygen, and for in vivo reaction the oxygen must be obtained from some other source than atmospheric oxygen, which was tyrosinase. Rothman's (1940) conclusions have answered this objection by demonstrating that

dopa can still be formed from tyrosine, in the absence of tyrosinase, if intracellular non-specific oxidation catalysts are present.

The final step in the process of pigmentation is the appearance of the pigment in the superficial layers of the epidermis to give the skin the characteristic tan color seen after exposing to artificial and natural ultraviolet radiations. This phenomenon is described by Edwards and Duntley (1939a, 1939b). They observed that as the erythema fades it is replaced by suntan, the transition from the one state to the other being almost imperceptible. Quantitative studies of the spectral distribution of the radiation reflected from tanned and unexposed skin indicate that their difference in color is due principally to the amount and position of melanin in the former. In normal untanned skin the melanin pigment is located chiefly in the cells of the basal cell layer. A few days after exposure to ultraviolet radiation, about the time suntan first makes its appearance, the pigment begins to migrate into the more superficial epidermal layers, eventually reaching the corneum. As a result, the basal cells may appear, after some days, to be almost free of melanin. The movement of melanin toward the surface causes the skin to present a darker appearance to the eye, even though the total amount of melanin is not increased at this early stage of suntan. Hamperl, Henschke, and Schulze (1939) found that the elaboration of new melanin after the old has moved into the more superficial layers of the skin is accomplished chiefly in the basal cell layer according to the

method described by Peck (1929, 1930, 1931), which was presented earlier in this paper.

Thus, like erythema, pigmentation may be readily explained on the basis of cell injury since it may develop following injury to the cells of the epidermis by other noxious factors. Lewis (1927) and Peck (1929) found that such factors as heat, chemical, or mechanical injury as well as ultraviolet light cause pigmentation, which is generally preceded by erythema. Blum (1941) claims it may be assumed that the migration of the pigment from the undamaged basal cells into the injured cells of the more superficial epidermis is due to some "tropic" action of the injured cells. The subsequent formation of new pigment in the basal cells may be explained by the action of tyrosine, elaborated by the injured cells, which is converted to dopa and then to melanin in the dendritic melanoblasts of the basal cell layer.

Darkening of the preformed pigment

However, all visible pigmentation is not solely due to the movement of the old melanin to the superficial layers of the epidermis, which is eventually followed by the production of new pigment in the basal cell layer. The other process which results in visible pigmentation was first called, by Hausser (1938), "darkening of the preformed pigment". This process was discovered by Lignac (1923) when he found that he could cause darkening of dead pigmented skin by ultraviolet radiation even though it was impossible for new melanin to be produced by this

reaction. Uhlmann (1930), when trying to confirm Hausser and Vahle's (1927) results that pigment production paralleled erythema production, noticed that pigmentation could be caused at 3,003 A.U. without the appearance of a preceding erythema although this type of pigmentation was not as strong as the type which followed erythema. Uhlmann's conclusions were confirmed by Luckiesh and Taylor (1939) who also observed that the tanning spectrum has a considerably longer wave-length limit than the erythema spectrum and that when applied in comparable erythemal dose, sunlight or carbon arc radiation, both of which are rich in wave-lengths longer than 3,200 A.U., produce a deeper tan than mercury arc radiation, which is weak in such wave-lengths. Thus, it seems evident that there is an additional process of pigmentation which supplements the tanning followed by erythema. This darkening of the preformed pigment when formed alone, gives a very light tan, but when it occurs in conjunction with the tanning process which succeeds erythema, it gives a much darker color to the skin than the tan after erythema would give per se.

The first intensive study on the darkening of the preformed pigment was done by Hausser (1938). She also foundthat this blackish coloration of the skin is brought about by wave-lengths longer than those of the sunburn spectrum. The action spectrum for this process extends from 3,000 to about 4,400 A.U., having a broad maximum at about 3,400 A.U. It may appear within a few minutes and passes its maximum within an hour after radiation, thus differing markedly in its time relationships from the pig-

mentation caused by the sunburn spectrum. About one thousand-fold greater dosage of radiation is necessary to elicit this response than is required for sunburn erythema and it is more pronounced in previously tanned skin. Hausser termed this phenomenon as "darkening of the preformed pigment" but gave no explanation as to the mechanism which caused it. Hamperl, Henschke, and Schulze (1939) have shown by histological examinations that the longer pigment darkening wave-lengths do not cause pigment migration to the superficial layers of the epidermis nor the formation of new melanin in the basal cell layer.

Henschke andSchulze (1939a, 1939b) observed, as did Hausser (1938), that a dark brown coloration of the skin is brought about by wave-lengths longer than those that produce erythema and so they too studied the effects of ultraviolet rays over 3,200 A.U. on the human skin. In the first place, they found that they could produce pigmentation without preceding erythema and thisresponse represents darkening of the preformed melanin. The shortest latent time was less than two minutes and the maximal pigmentation occurs thirty minutes to one hour afterexposure to the radiations. Additional exposure to ultraviolet rays under 3,200 A.U. had no influence on the effect and histological studies showed that, in contrast to erythema, there was no inflammatory process. It differs in several respects from the primary melanization that follows sunburn. The action spectrum for pigment darkening extends from about 3,000 A.U. to 4,200 A.U. with a broad maximum near 3,400 A.U. These figures are very similar to those arrived at

by Hausser (1938). The action spectrum for melanization of the epidermis is the same or very similar to the erythema spectrum. Hence, in contradistinction to erythema and pigment formation, pigment darkening is readily brought about by sunlight passing through window glass which removes wave-lengths shorter than 3,200 A.U. Pigment darkening may appear within the first few minutes of exposure to sunlight whereas melanization is not manifest until a few days later. Several hundred fold greater dose of radiation is required to produce pigment darkening than is required to cause erythema and melanization. Pigment darkening is more pronounced in skin that has been previously sunburned and still retains traces of suntan, whereas melanization is most pronounced in skin not previously sunburned. Pigment darkening does not occur if oxygen is removed but erythema and melanization are not effected by this treatment.

Miescher (1939) has also described pigment darkening by high intensities of long ultraviolet wave-lengths characterized by immediate appearance, as contrasted with pigmentation produced by shorter wave-lengths which has a considerable latent period. The first type of pigmentation is due to transformation of a paler form of pigment into a darker form by absorption of oxygen; warmth as well as long ultraviolet rays can mobilize the required oxygen. The second type of pigmentation is due to increased accumulation of pigment. Miescher suggests that the absence of the latent period in pigment darkening may indicate direct action on the blood vessels, which are reached by these longer waves but not appreciably by those shorter than 3,200 A.U.

Thus, pigmentation resulting from ultraviolet light has two more or less independent phases, each with a different action spectrum: (a) formation of new pigment has the same action spectrum as erythema of sunburn and is a sequel to that process; and (b) darkening of pigment already present in a bleached form upon exposure to wave-lengths between 3,000 A.U. and 4,200 A.U., the action spectrum having a rather flat maximum at 3,400 A.U.

The mechanism which causes pigment darkening was first explained by Miescher and Minder (1939). They confirmed the findings of Henschke and Schulze (1939a, 1939b) and also offer evidence that this process is identical with the darkening of dead pigmented skin brought about by heat and ultraviolet radiation, as found by Lignac (1923). All three phenomena occur only in the presence of oxygen and they believed that a common mechanism was involved. This, they suggest, is the oxidation of pigment already present in the skin in a reduced leuco-form and this leuco-form of melanin might well be the light absorber for this process. It is, thus, a reversable process quite independent of the formation of new pigment.

Many additional investigations have borne out Miescher and Minder's theory that pigment darkening iscaused by the oxidation of reduced melanin. Hamilton and Hubert's (1938) studies on three castrated and four hypogonadal males have shown that the typical pasty sallow color was lost and that tanning occurred after administration of male hormone. Exposures to ultraviolet radiations over a period of five months caused erythema but this was not followed by melanization, as normally happens, nor

was pigmentation seen to result afterexposures of longer wavelengths than those which produce erythema. However, after administration of the male hormone to the exposed areas, a tan formed and they believed that this tanning was due to the oxidation of reduced melanin. Miescher and Minder's hypothesis also has received support from Figge (1939) by a demonstration that melanin behaves as an oxidation-reduction indicator; the oxidized form being dark, the reduced form bleached. He observed that sodium hydrosulfite reduces black solutions of melanin, which absorb 70% of the light, to a tan solution that absorbs only 25% of the light. Potassium ferricyanide reoxidizes this to a black solution. Edwards, Hamilton, Duntley, and Hubert (1941) also observed, as did Hamilton and Hubert (1938), that ultraviolet irradiations of the untreated castrate's skin did not stimulate the production of melanin but did induce the formation of a large quantity of melanoid, the reduced form of melanin. In a castrate, in the cutaneous areas, there is found a large venous bed with a great amount of reduced hemoglobin because of the dilated condition of the veins, which is peculiar to these men. Consequently, the rate of flow of blood in the cutaneous areas of these individuals is slower than that of normal men due to the dilated venous bed. However, upon administration of the male hormone, testosterone propionate, this condition was alleviated and the large amount of reduced hemoglobin was replaced by oxyhemoglobin because of the increase in circulation through the venous beds. As a result, the melanoid formed by irradiation wasoxidized to melanin and the characteristic pale color of the castrate's

skin was replaced by the normal tanned hue. This experiment also demonstrates that tanning may be due, in part at least, to the oxidation of reduced melanin.

Thus, it seems to have been proved conclusively that in addition to the pigmentation following erythema there is another process of tanning. This type of pigmentation is brought about by ultraviolet wave-lengths that are longer than those found in the erythema spectrum. These longer rays activate an already present reduced form of melanin so that it is converted to an oxidized form, without any latent period, which results in the appearance of this second type of pigmentation. Miescher (1939) and Edwards, Hamilton, Duntley, and Hubert (1941) believe that this oxygen is supplied by the oxyhemoglobin of the blood stream since these longer rays are able to penetrate to the cutaneous blood vessels. Until some other method of oxidation of the reduced melanin is proven, such as possibly by a specific oxydase, this explanation will have to stand as accepted in order to provide a reasonable working hypothesis for future experimentation along this line.

Development of protection against sunburn

It is common experience that skin which has been exposedto sunlight becomes less susceptible to subsequent irradiations. The first experiments to determine the cause of the acquired immunity of skin to become more resistant to sunburn after an initial exposure to sunlight were performed by Finsen (1900). As a result of his findings, a false concept was engendered that still

persists. It is commonly believed that the action of pigment is to filter out ultraviolet rays. This seems so obvious that it has been assumed that the decrease in susceptibility to erythema is due to the formation of pigment. Finsen covered a part of the arm with India ink, leaving the remainder uncovered, and exposed the whole arm to sunlight for three hours. The uncovered part was sunburned, but the covered part remained unchanged. After several days, when the sunburned area of the arm had become tanned, he again exposed the arm to sunlight but without any covering. Following this second exposure, that area developed sunburn which had been protected by the ink during the first exposure, whereas the previously sunburned areas were scarcely affected. Finsen attributed the acquired immunity to screening by pigment developed as a result of the first exposure, which he thought mitigated the effects of the sun's rays by absorbing them. The idea that suntan directly protects against sunburn was generally accepted at this time, and it is still widely held today.

However, studies of the position of the pigment in the skin and the locus of action and penetration of the skin by sunburn radiation make it improbable that the pigment can offer a great deal of protection. Moreover, skins which have no trace of pigmentation may lose their sensitivity to light after frequent exposures. With (1920) and Meyer (1924) have very convincing proof that immunity to ultraviolet radiations may be acquired without pigmentation. They made their studies on the skin of vitiligo patients (these people lack pigments of any sort in the epidermis)

and found that exposure of the non-pigmented areas of the skin caused increased resistance to further exposures of ultraviolet radiations. Thus, persons who completely lacked pigment in the skin could lose their sensitivity to ultraviolet light. Nevertheless, it cannot be categorically denied that pigment plays some part in the protection of skin, although perhaps a minor one.

Since it was evident that some factor other than pigmentation was mainly responsible for immunity to sunburn, many experiments were performed to determine the nature of this other agent. Almost immediately the investigators found themselves on the right path when Perthes (1924) believed that immunity to ultraviolet radiation was developed by the epidermal cells themselves. He based his belief of cellular immunity on the apparent rapidity with which sensitivity to sunburn decreased after exposure, as he noted that a second dose of ultraviolet radiation produced less effect when it fell on a spot that had been already exposed, even so short a time as one hour previously, although no pigment had had a chance to form. Guillaume (1926, 1927) opposed the view of Finsen when he found that lesions produced by the ultraviolet show that the damage is outside the pigment, that is between the screen and the source of radiation. Consequently, he was the first to point out that the thickening of the corneum or horny layer of the epidermis might be the principal protective factor since he observed that repeated irradiations thicken this layer, and the thickening parallels increased resistance. This thickening is a result of damage to the underlying prickle

cell layer and it decreases the intensity of the sunburn wavelengths which reach these cells, and, hence, the sensitivity of the skin to sunlight. Measurements of the transmission of sunburn radiation by the skin show that a considerable degree of protection would be afforded by relatively slight thickening. Guillaume disagrees with Bloch and Schaaf (1925), who claim that melanin absorbs the sunburn radiation strongly and should afford a considerable degree of protection whether in the corneum or in the Malpighian layer, where it may act as an internal filter. He believes that the only function of the melanin is to protect the organism against excessive visible radiation and perhaps, to some extent, infrared since the pigment is located principally in the basal cell layer of the epidermis, whereas the findings show that the cells primarily affected in sunburn are chiefly the prickle cells which lie superficial to most of the pigment. This arrangement of the pigment is characteristic of white skin; but in Negro skin it is more evenly distributed throughout the epidermis.

Another hypothesis was put forth by Hausmann and Spiegel-Adolf (1927) who suggested that coagulation of proteins in the skin may serve as a protective device since irradiated proteins lose their transparency in the region of the sunburn spectrum. This theory has been discarded because of the lack of sufficient evidence and also because the thickening of the corneum hasbeen reported by many investigators to be of most importance in protective action.

At first, Schall and Alius (1927) accepted the idea of

Hausmann and Spiegel-Adolf (1927). They believed that immunity may have been caused by changes in the colloidal chemistry of the skin resulting in a change in its transparency to radiation. The effects of single continuous doses of ultraviolet radiation were contrasted with those obtained following intermittent doses; Ordinary summation effects could not be demonstrated and increase in redness of the skin was not proportional to increase in dosage. Following all exposures there is a decrease in sensitivity which appears after a certain period of time and then disappears again. The immunity is dependent upon the intensity of the exposures and the frequency in which exposures are made since subthreshold exposures produce less accustoming of the skin to irradiations than to more intense but less frequent doses, or single doses of the same total value. Because they could not always demonstrate the presence of pigment after the accustoming had occurred, they thought that invisible amounts may be responsible for protection against radiation effects and yet, in some cases, despite a heavy pigmentation, no protective action was seen. Thus, they concluded that pigmentation was only one factor in immunity and the other factor may have been the change in the colloidal chemistry of the skin so that the transparency to radiations was decreased. However, Schall and Alius (1928), upon further investigation, rejected Hausmann and Spiegel-Adolf's (1927) theory and accepted the point of view proposed by Guillaume (1926, 1927) that thickening ofthe corneum was the important agent in protective action. They noticed that following quartz mercury lamp irradiation, human skin developed a height-

ened erythema threshold for subsequent irradiation. This effect was not correlated with the amount of pigment as the decrease in sensitivity disappeared after fifty to sixty days even when pigment remained. The maximum reduction of sensitivity of sunburned skin appears about one week after the exposure and normal sensitivity is regained in about fifty to sixty days. Thus, they concluded that thickening of the epidermis might reach its maximum in the former period, and normal thickness regained in the latter, suggesting that the corneum thickening is the major factor in conferring immunity since suntan, on the other hand, may persist for many months after immunity is lost.

That pigmentation is not the major factor in the acquired immunity to ultraviolet radiations was also demonstrated by Keller (1928) and Keller and Rein (1928). They believed that tolerance to radiation is a consequence of changes in the outermost layers of the epidermis which show themselves by polarization changes rather than by direct radiation and it is not a result of pigmentation since the latter occurs below the point of attack of the ultraviolet rays in the horny layer of the epidermis. Polarization of the skin is a measure of the permeability ofthe cell membrane; it was measured as a direct current resistance and found to be lowered by ultraviolet erythema. However, of greater importance is the decrease in polarization just before the formation ofan erythema and especially during the pigmentation which results. This proves a decrease in the permeability of the cell membrane of the upper skin layers which is probably the reason for the light protection afforded by the

irradiated skin. The lowered permeability is mainly due to the increased thickness of the skin as additional dead cells are added to the superficial layers.

Lovisatti (1929) also did experiments to determine the importance of the thickening of the corneum. He carried out two series of experiments, one on normal subjects and the other on an albino. Erythema was induced in both by ultraviolet radiations and both acquired tolerance at about the same time under daily irradiations. However, under irradiations spaced ten days apart, the normal subject acquired tolerance toward the sixth or seventh day while the albino failed to acquire it at all. Also, the albino lost tolerance in fifteen days of non-irradiation; the normal subject did not respond to irradiation with erythema until the twenty-fifth day. Lovisatti concludes that the skin protects itself from ultraviolet rays by two responses, of which the first and most important is the thickening of the stratum corneum, which absorbs the rays, while the second is the ascent of the cutaneous pigment toward the superficial layers to aid in the absorption of the rays.

Very intensive studies on the relative importance of pigmentation and thickening ofthe corneum to skin tolerance were made by Miescher (1929, 1930, 1939). Radiation with ultraviolet rays causes hyperkeratosis and pigmentation. Both keratin and pigment are effective protectors against ultraviolet radiation because they absorb the ultraviolet rays to a great extent; but the pigment, because of its location in the deeper layers of the skin, is presumably of less importance as a protective

mechanism. The protective action of the skin against ultraviolet light depends primarily on the thickening of the epidermis which protects the underlying tissue by scattering and absorbing the light. Ultraviolet is absorbed by the corneum in proportion to the thickness of this tissue which explains the variations of sensitivities to the light of different body regions. Histological studies of the reaction produced by light shows a uniformly progressive penetration proportional to the dose and only influenced by differences of the corneus layer. Resistance to the reaction of light is built up by increasing the thickness of the corneum and, hence, it is closely connected with increased keratin formation. Absorption is chiefly due to phenylalanine, tyrosine, and cystine contained in the keratin. In investigating the extent to which keratin absorbs ultraviolet rays, Miescher found that an increase in thickness of the horny layer ofonly eight to nine micra reduces the effectiveness of short ultraviolet rays to one-half.

Miescher (1932) also made studies on the differences of white and negro skins to ultraviolet after Hausser (1928) found that the action spectrum for a negro was the same as for his white subjects although the threshold of the negro was much higher. In white persons, increasing tolerance to ultraviolet radiation ismainly due to hyperkeratosis and the protective action ofthe pigment is almost negligible because pigmentation in whites occurs only in the basal cell layer and, except for very high doses, ultraviolet rays do not penetrate to such depths. In the negro, however, the pigment ofthe skin offers a

considerable protection against sunburn radiation since it is more uniformly distributed throughout the epidermis and damage to the skin from ultraviolet rays is, therefore, in the negro confined to the uppermost layers of the epidermis except in extremely high doses. Miescher claims that the absorption spectrum for melanin is almost uniform in the sunburn spectrum so that its presence in the superficial layers should not markedly alter the shape of the action spectrum, although decreasing the general sensitivity as in the negro. It is also possible that the corneum is thicker in the negro race than in the white.

Various studies have been made on other protective measures against sunbrun that are not included in pigmentation and thickening of the corneum. Kofman (1933) explains that the protection afforded by a coat of oil is due to the fact that the oil increases the diffusion of ultraviolet about ten times and it has been established that increased diffusion decreases the amount of transmission of the ultraviolet through the epidermis. Crew and Whittle (1938) found that human sweat, by virtue of its partial opacity to ultraviolet light, affords some slight protection to the skin against those rays which are capable of producing erythema. It is computed that a sweat film one millimeter thick transmits only 27% of solar radiation effective in producing erythema. However, Blum and Terus (1946b) do not agree with Crew and Whittle as they observed that sweating did not appreciably affect the erythemal threshold and wet skin is neither more nor less prone to sunburn than dry skin. Blum, Eicher, and Terus (1946) are in concordance with previous au-

thors that the best natural protection against sunburn is by thickening of the corneum, so that absorption is increased, and the presence of melanin is of less importance. They claim that the best artificial protectors are those that scatter light and, in view of this fact, the best sunburn preventive is titanium dioxide compounds as they are the most effective in scattering light. On the whole, they find that it is difficult to measure the good of any artificial preventive because of so many variables involved. The major variable, and the one having the greatest effect on quantitative sensitivity, is the factor that the thicknessof the corneum varies with individuals. Altogether there are three variables which make conclusive findings difficult to determine: (a) the intensity and spectral distribution of the sunburn-producing radiations in sunlight; (b) the erythema threshold of the individual; and (c) the thickness of the layer of the preventive. For these reasons it is just about impossible to arrive at any kind of conclusion concerning the effectiveness of different artificial sunburn-protective devices.

While all evidence indicates that thickening of the corneum is the major factor determining the relative immunity to sunburn following exposure, the possibility that melanization and other factors play a role cannot be completely denied. The amount of radiation transmitted by the corneum is a function not only of the thickness but also of both absorption and scattering. Thus, the transmission of the corneum might be altered in three different ways: (a) by changes in the absorbing component, e.g.,

the accumulation of melanin; (b) by thickening; and (c) by alteration of the scattering characteristics. As pointed out, the second of these factors is no doubt the most important.

Normal variations in susceptibility to ultraviolet

It is common observation that certain individuals are more susceptible to sunburn than others, so that it is difficult to determine the norm for the erythemal threshold. While attempting to establish a norm and also identify the light absorber, Schall and Alius (1926) showed that the rate of development and fading of erythema is different for different individuals and this renders the erythemal threshold an arbitrary index at best. It also makes the identification of the light absorber relatively inexact. Schall (1928) investigated the course of the erythema reaction produced by ultraviolet light upon the skin of three hundred persons by means of an "erythema-meter", which consists of various scales of reddening and takes into account the natural color of the skin. In spite of the precautions taken in the determination of the course of the erythema reaction, wide variations existed in different individuals under the application ofidentical doses. Although the qualitative and quantitative course of the reaction was different in each individual, certain types of reactions could be collected into groups. There is usually a rapid increase of the skin reaction to a maximum, which occurs in 60% of the cases between the fifth and ninth hour after treatment. In most cases a definite wave form of the reaction could be observed. The latent period va-

ried from one to seven hours with an average of two hours. Hausser (1928) also noticed that the relative rate of erythema varies for different individuals when he tried to determine the erythema action spectrum. The spectrum remains constant for all people, even negroes, but the rate of development of the erythema varies for almost every person. Varying sensitivities to later exposures were observed by Ledermann and Meyer (1926) when irradiations lasting from two to one hundred and ten seconds, in intervals ranging between forty-five and fifty minutes and between one and two and one-half hours, produced in some an increased sensitivity and in others a decreased sensitivity to later exposures.

Besides differing for different individuals, the erythemal threshold may vary on different parts of the body of the same person. This makes the determination ofthe erythemal threshold even more difficult for different investigators may use skin from various body parts in the belief that the threshold will be the same all over for any one person. Meineri (1937) made histological examinations of strips of skin from the back and legs of the same person irradiated with ultraviolet light and found that twenty-four hours after irradiation, a reactivity of various degrees manifested chiefly by a lymphocytic infiltration on the back while the infiltration on the legs consisted of polynuclear neutrophils. The serum extracted from the skin of the back contains a larger quantity of albumen than the serum extracted from the skin of one of the legs (in average, a difference of 1%). This indicates that the skin in its different

parts has different properties and tendencies, probably related to the needs of each region. This can be tied into the fact that erythema and pigmentation are greater on the back than on the legs but no reason is advanced as to why this is so outside of the fact that there are physiological differences in the back and legs as caused by ultraviolet irradiations. Since ultraviolet light isabsorbed by the corneum in proportion to the thickness of this tissue, Miescher (1930) offers this as an explanation to the variations of sensitivities to light of different body regions as histological studies have shown that different parts of the body vary in the thickness of the corneum.

Ellinger (1932b) has made an extensive study of the threshold dosage for erythema production, which provides very interesting data. The method which he employed was to expose small areas of the skin to doses of mercury arc radiation of the same intensity but different durations, and to examine the exposed areas for erythema twenty-four hours later. By using equal increments of the irradiation period, the number of areas which show erythema may be taken as a measure of the sensitivity of the skin to light. The data from this type of measurement, when considered statistically, show changes with seasons of the year (sensitivity being least during the summer), with age of the individual, and with other factors. He finds that blonds are more sensitive, as a rule, than brunettes, and light or reddish blonds more sensitive than darker blonds. There are also statistical variations among women with relationship to the menses and pregnancy. Ellinger (1932c) finds that individuals who ex-

hibit certain characteristics - "vegetative stigmatics" - are much more sensitive than the average. The individuals having these characteristics are assumed to have mild hyperthyroidism, and Ellinger believes that sensitivity of the skin to sunburn radiation is related, in some way, to the activity of the thyroid gland.

Additional figures on variationshave also been presented by Laurens (1939) who confirms many of Ellinger's above findings. Blonds are 40-170% more sensitive than brunettes, men are 20% more sensitive than women, and persons between twenty and fifty are more sensitive than younger or older. Maximum sensitivity occurs in March and April, and in October and November. Persons with unstable nervous systems, overactive thyroid glands, elevated blood pressure, an increased number of open capillaries in the skin, or active tuberculosis show greater sensitivity. Accident increases sensitivity and sensitivity increases at the menses, being higher on the first day of the cycle and then declines to normal. There is increased sensitivity after the second month of pregnancy which begins to diminish at the seventh month until birth. Thus, erythema and pigment formation depend on individual factors such as race, coloring, constitution, and body function.

Blum and Terus (1946b) published a paper concerned with various other physiological and physical factors which determine the erythemal threshold and cause it to differ among individuals. Long ultraviolet waves can change the threshold by directly affecting the cutaneous vessels and altering their sensitivity to

the dilator substances. The thickness of the corneum affects transmission since anything that increases scattering will decrease transmission. The Malpighian layer also varies in thickness and this too has its effects on transmission. The threshold of a given individual differs from time to time due to nervous and hormonal reactions. Thus, there can be no standard erythema since the factors governing susceptibility to erythema vary among individuals. Under such circumstances it is difficult to state where normal sunburn ends and hypersensitivity begins. It is necessary to recognize a wide latitude between the extremes of what is considered normal.

Variations in the ultraviolet intensity of sunlight

There are many natural factors concerned with sunlight itself that make accurate measurements of erythema a difficult task. The various intensities of sunlight and agents which can have serious effect on these intensities have been made the study of Coblentz, Stair, and Hogue (1933). That part of sunlight which produces sunburn, i.e. wave-lengths shorter than 3,300 A.U., is a very small and variable fraction of the total as it amounts to only 0.2%. The short wave-length limit is dependent upon such factors as the time of day, season of the year, and locality. At no time of the year is this limit appreciably shorter than 2,900 A.U., and frequently is not longer than 3,300 A.U., even at midday; in the latter case the sunlight has no sunburn-producing power. Smoke is very effective in absorbing the short wave-lengths of sunlight and thus in prevent-

ing sunburn; but, on the otherhand, water vapor allows these wave-lengths to pass, so that it is often possible to be badly sunburned on a cloudy day. Clouds over the vicinity of a large city may contain a considerable amount of smoke, and should be much more effective in filtering out the sunburn radiation than clouds over a rural area, or particularly over the sea. This probably accounts for the fact that sunburn frequently occurs at the seashore on overcast and foggy days, particularly when an onshore wind removes any traces of smoke. Water, snow and ice are good reflectors for the sunburn radiation, and sunburn is very common close to the sea or other bodies of water, or to snow or ice, e.g., above glaciers. The terms "snowburn" or "glacierburn" are often applied to the severe sunburn that may occur after exposure on snowfields or glaciers. All of these variable factors in the character of the incident sunlight, together with the variations of the individual threshold, give sunburn a capricious behavior which frequently mystifies the scientific as well as the popular mind.

Antirachitic action ofultraviolet

Beside the erythema and pigmentation produced by ultraviolet radiations on the skin, it has also been found that certain ultraviolet wave-lengths are very effective in curing rickets by photochemical alteration of certain sterols located in the skin. This is a primary photochemical reaction that occurs in all human skin in conjunction with erythema and pigmentation. Bunker and Harris (1937) investigated the definite

spectral zones for the first time to show the antirachitic action of each. Transformation in vitro of 7-dehydrocholesterol into its isomer vitamin D seems closely related to the process which takes place in human skin. This transformation of the precursor is done by the action of ultraviolet radiations shorter than 3,200 A.U. The principal mercury lines between 2,537 and 3,025 A.U., inclusive, were demonstrated to have antirachitic properties with the greatest value obtained at 2,967 A.U. Adjacent lines immediately below and above this region were inactive. The different values of the various spectral lines are probably due in part to differences in absorption of the various wave-lengths by theskin. They are likely due in part to the absorption spectrum of the provitamin. On a quantum basis the antirachitic effectiveness of the different wave-lengths is not the same. Knudson and Benford (1938) also attempted to determine the action spectrum for the antirachitic action of ultraviolet radiations and obtained figures that were not entirely in agreement with those of Bunker and Harris. They found that light ranging from 2,653 to 3,128 A.U. was most effective and the spectral line of 2,804 A.U. was the most beneficial of all. Measurements of this action spectrum agree quite well with the absorption spectrum of provitamin D (7-dehydrocholesterol).

The above authors point out that, although this process has the same approximate long wave-length limit as sunburn, it does not necessarily indicate a relationship between the two phenomena which may be regarded, from a physiological point of

view, as coincidental. Thus, while the data of Bunker and Harris (1937) and Knudson and Benford (1938) differ to some extent, both indicate that the photochemical process is definitely not the same as that of sunburn. Both groups find a relatively high antirachitic effectiveness at 2,800 A.U. where the erythema spectrum shows a decided minimum. This minimum in the erythema spectrum is due to the strong absorption of these wave-lengths by the superficial layers of the skin, particularly the corneum. These investigators claim that because this minimum is not reflected in the antirachitic action spectrum indicates that the formation of vitamin D takes place much more superficially than the changes which lead to the erythema, possibly on the surface and in the corneum. This seems very logical since, if the photochemical reaction occurred at any significant depth, it would be subjected to modification from screening by the more superficial layers of the epidermis as occurs in erythema of sunburn. Further evidence as to the fact that the provitamin D is located in the most superficial layers of the epidermis has been given by Helmer and Jansen (1937). They found that washing removes fractions from the human skin which ultraviolet rays can convert into antirachitic agents. When the oily fraction was removed from non-irradiated skin then irradiated and fed to experimental animals, healing was induced. Irradiation prior to extraction from the skin seemed to yield a more potent product.

Carcinogenic action of ultraviolet

One aspect of ultraviolet light that has received consid-

erable attention is its ability to produce tumors in the skin. Close relationship between the sunburn and carcinogenic mechanisms is suggested by the fact that both have the same long wave-length limit at 3,200 A.U. Hence, it seems reasonable to relate the carcinogenic mechanism to the same fundamental injurious action on cells that is associated with sunburn, and is characteristic of ultraviolet radiation of wave-lengths shorter than this limit. Unna (1894) was the first to propose the idea that prolonged exposure to the weather may produce, in the exposed skin, changes which not infrequently terminate in cancer. He describes "Seemanshaut" (a condition common to sailors) as a precancerous condition attributable to continued exposure to sunlight. Many tumor-like growths about the face and neck of a thirty-five year old South African, who had been working in the sun for fifteen years, were seen by Sheild (1899) and he also attributed these growths to the action of the sun on the skin. That exposure to sunlight is an important factor in causing cutaneous cancer was also noted by Hyde (1906) who found that disorders of this type are more frequent in adults than in children and reaction to the play of actinic rays of light on the skin are chiefly determined after the middle periods of life have been reached. Physiological pigmentation of the skin in the colored races seems to furnish relative immunity to that organ against cancerosis and the colored races apparently suffer less than the whites from cancer of the skin. This relative immunity may be due to the protection from the actinic rays of light furnished by the pigment of the integu-

ment. He found that whites exhibit cancer on the face, neck, and hands more than on any other part of the body, and that it is especially common among agricultural workers who are constantly exposed to sunlight. Cheatle (1925) microscopically examined skin that had been bronzed therapeutically by exposure to sunlight and noticed a very marked mitosis occurring in the epithelial cells situated above the basal cell layer. He points out that the skin changes of an old countrywoman, which he once attributed to life wear, is, no doubt, mainly due to the sunlight factor of life wear. He also found that white people in tropical countries and gardeners frequently are subjected to squamous epithelioma in the areas of the skin continually exposed to sunlight. Cheatle finally mentions the caution needed in the application ofsunlight and ultraviolet rays for therapeutic purposes since he saw a lesion having recently been exposed to ultraviolet light getting worse because of increased mitosis. Young and Russell (1926) observed that cutaneous cancers are more prevalent in fishermen, bargemen, agricultural laborers, farm servants, farmers, gardeners, and any other people who work in the sun.

Several investigations have been made to determine the incidence of cutaneous cancer on various parts of the body. All of these studies have shown that those regions most exposed to sunlight have the largest amount of cancer. Lacassagne (1933) found that in 91% of 1,626 cases reviewed, the tumors occurred most frequently on the face; and he inferred from this that sunlight is the principal agent responsible for such neoplasms,

since the face is probably more exposed to the sun's rays than any other part of the body. A very extensive study extending over a period of several years was made by Roffo (1929, 1931, 1936) and Roffo and Pilar (1930a, 1930b). Among 5000 patients attending the Cancer Institute of Buenos Aires none showed cancer on any part of the skin covered by clothing. The frequency of cutaneous cancer for regions exposed to the sun showed 95.51% on the skin of the face and 3.07% on the skin of the back of the hands. In the face the parts most often affected are those most prominent and exposed; 61% on the nose, 18% on the cheek, and hardly any on the forehead. Men have 70.9% of the cancer and women 29.1%. This lower incidence in women is due to the fact that they use powder to cover their skin. The majority of the women having cancer were countrywomen who did not bother to powder their skin. The lesions were most frequent in workmen, farmer, and planters who have to expose themselves to the sun all day. All cases were among the white population and none were found in natives, negroes, or mulattoes. These patients at first exhibit diffuse erythema followed by pigmentation. The pigmented zones get more pronounced and hyperkerotic; then they ulcerate and become cancerous.

All of these previous studieshave served to demonstrate that some factor in sunlight is responsible for the appearance of cutaneous cancer. The first experiments to determine which part of the solar spectrum was effective in causing epithelioma were performed by Findlay (1928). By exposures of mice to ultraviolet light for a period not less than eight months it

is possible to produce papillomata and milignant epitheliomata of the skin. Findlay (1929) also produced these conditions in the rat but where it only took eight months to get tumors in the mouse, it required twenty-one months to arrive at the same condition in the rat. In man, it takes fifteen to twenty years to cause skin cancer by light. It is, therefore, probable that the time required for the induction of cancer is dependent on the particular species involved rather than on the nature of the stimulus. The time necessary for the production of cancer thus increases with the length of life of the species. Purschar and Holtz (1930) were successful, with great regularity, in producing skin cancers on rats by ultraviolet irradiations; but it only took them thirty-seven weeks to observe cancer in all the animals used whereas Findlay needed twenty-one months. They accounted for this considerable difference in time as due to the fact that they irradiated the ears while Findlay used the back. The ears have a much thinner corneum than the back and, as a result, more of the ultraviolet rays are able to penetrate deeper and have a greater effect on the underlying tissues.

Now that it had been established that the ultraviolet rays of the sun were the cause of skin cancers, it remained to be determined which of the ultraviolet wave-lengths were most effective. The methods used by Roffo (1934), Beard, Boggess, and von Haam (1936), and Rusch, Kline, and Baumann (1941) were approximately the same and the conclusions reached were in agreement. In determining the active wave-lengths that are responsible for skin tumor, they all employed a mercury arc lamp

and a window glass of ordinary thickness. Two types of mercury arc lamps were used: one was an intermediate pressure lamp which gives off ultraviolet waves longer than 2,250 A.U.; and the other was a low pressure lamp which emits virtually all of its radiation at a wave-length of 2,537 A.U. It was apparent from their results that only mercury lines of wave-lengths 3,130 A.U. or shorter cause tumor production since the mercury arc radiation that passes through window glass is ineffective. Window glass of ordinary thickness removes all lines shorter than 3,200 A.U. and the intermediate mercury arc emits relatively little energy in the longer wave-lengths as compared with sunlight. This might account, conceivably, for failure to produce tumors with mercury arc radiation passing through window glass. However, mercury arc radiation that passed through a Corex D filter was about as effective as unfiltered radiation. A Corex D filter eliminates virtually all the energy of wave-lengths shorter than 2,967 A.U. and yet tumors were induced. The low pressure mercury arc did not induce tumors and, thus, it seems that the short wave-length limit for tumor production is in the region of 2,900 A.U. Since transmission of ultraviolet light through the epidermis falls off rapidly around 2,900 A.U., it suggests that the short wave-length limit may be set by this factor. Next, a tungsten filament was used as their source, which emits most of its radiation in the visible and the infrared, and no tumors were formed. Beard, Boggess and von Haam also used a General Electric SI lamp which emits mercury arc radiation in addition to the radi-

ation from the tungsten filament, and in this case tumors were produced. Total sunlight was effective in producing skin tumors but sunlight passing through window glass was not. The shortest wave-length in sunlight is about 2,900 A.U. and since window glass was used, the longest effective wave-lengths of sunlight must be in the neighborhood of 3,200 A.U. This agrees with the findings for the mercury arc. Thus, as a result of these various investigations, it has been shown that the tumor-producing wave-lengths in sunlight are limited to a very restricted portion of the whole, namely, those wave-lengths lying between about 3,200 A.U. and the short wave-length limit at 2,900 A.U.

These experiments clearly indicate that the action spectrum for cancer production does not agree with the erythema, since no tumors could beproduced with radiation of wave-length 2,537 A.U. with the low pressure mercury arc although this is quite effective in causing erythema. Blum (1940) points out that in sunburn the primary change is in the epidermis, whereas for cancer production it may be necessary to affect deeper layers also, i.e., the corium. In this case the epidermis as a whole may be regarded as a filter and, as a result, the action spectrum might virtually be restricted to only those wave-lengths longer than about 2,800 A.U. Such an explanation receives some support from the fact that sarcomas form a considerable part of the tumors produced by ultraviolet radiation in the laboratory. The pathological features of tumors of the external ear of mice induced by ultraviolet radiation were observed by Grady, Blum, and Kirby-Smith (1941) to show that the

predominant type of tumor was a fibrosarcoma. Less frequently combinations of fibrosarcoma and squamous carcinoma occurred, while only three instances of squamous carcinoma alone occurred.

However, caution must be used at this point in carrying over the evidence from experiments on rats and mice to the problem of cancer in man because, in contrast to rats and mice, malignant tumors of the skin of man virtually all arise from the epidermis. This apparent lack of agreement between the case of man and that of the mouse is explainable in terms of differences in penetration of the ultraviolet radiation into the skin of the two species. That ultraviolet radiation may induce tumors in a variety of tissues depending upon their susceptibilities and limited by the penetration of the radiation was determined by Grady, Blum, and Kirbey-Smith (1943) by examining the ratio of carcinomas to sarcomas. A difference in response of epidermal and connective tissue elements was demonstrated by the fact that dose per se did not affect the ratio, whereas the interval between exposures had a marked affect, the ratio increasing with the frequency of exposure. Kirbey-Smith, Blum and Grady (1942) found that in man those wave-lengths shorter than 3,200 A.U. are virtually all absorbed in the epidermis, and it is here where only epithelial cells are present, that human cutaneous cancer appears. In the mouse, on theother hand, this radiation penetrates much deeper because the epidermis of the rodents is much thinner than that of man. As a result, this radiation reaches the connective tissue and other non-epithelial tissues, accounting for the high proportion of sar-

comas found among the tumors induced by ultraviolet radiation in these animals. They also suggest that the importance of melanin in this case must not be completely ruled out as the melanin must function effectively in preventing penetration of the radiation below the epidermis, which may be of considerable importance in determining the sight of cutaneous cancer.

Blum (1940) presents five major lines of evidence which support the concept that sunlight can cause cancer of the skin: (a) cancer of the skin occurs principally on the parts of the body most exposed to sunlight (about 95% on the face and hands); (b) cancer of the skin ismore common among outdoor than among indoor workers; (c) the incidence of cancer of the skin is greatest among regions of the earth that receive high insolations, for example, in Australia the majority of skin diseases are rodent ulcers and epitheliomat which may be correlated with irritating action of the solar rays as accentuated by the relatively low humidity of this atmosphere (Lawrence, 1928); (d) cutaneous cancer is less prevalent in negroes than in white races presumably because the former are less susceptible to sunlight; and (e) cancer of the skin of laboratory animals (mice and rats) can be induced by exposure to ultraviolet radiations.

SUMMARY

Biologic reactions of the skin to ultraviolet radiations occur in the spectral range of 2,500 to 3,300 A.U. where the absorption of protoplasm is so great that the penetration of these waves into the body is very slight. A layer of skin 0.1 mm. thick is capable of absorbing almost all radiations in this range. The degree of transmission varies with different spectral lines, with a maximum absorption at about 2,800 A.U. and a second smaller maximum around 2,500 A.U. However, the different wavelengths are not all absorbed with the same equality in the skin as various layers of the epidermis exhibit characteristic absorption bands. At 2,750 A.U. practically all radiation is absorbed in the corneum and granulosum; and on each side of this band, rays are able to penetrate to the germinativum and corium. Below 2,500 A.U. the absorption of the horny layer increases so rapidly that no rays shorter than 2,400 A.U. reach any living part of the epidermis.

The action spectrum for erythema and pigmentation ranges from 2,400 A.U. to 3,250 A.U., with a sharp maximum at 2,970 A.U., followed by a sudden drop at 2,800 A.U., and then a rise to another smaller maximum at 2,500 A.U. The sudden decrease in effectiveness at 2,800 A.U. may be attributed to the fact that there is a maximum of absorption by the superficial layers of the epidermis in thisarea so that none of these rays are able to penetrate to the lower living cells and elicit a photobiologic reaction. The cells most affected by this action spec-

trum are found in the stratum spinosum, as histological examinations show that the prickle cells are the first to show degeneration following irradiations. Since wave-lengths on each side of 2,800 A.U. are able to penetrate to this layer, it may be concluded that the initial reactions to sunburn radiation occur here.

Between the periods of exposure and the first appearance of a just perceptible erythema, there is a considerable time lag called the latent period. It is during this time that the cells receiving the radiationsundergo reactions which result in the release of substances that cause the erythema. The exact nature of this latent period is still unknown, since it has a temperature coefficient unlike any substance that also has a latent period following irradiations.

The nature of the true absorbing substance which initiates the erythemic response has yet to be determined since both proteins and nucleic acids exhibit characteristic absorption within the erythemal action spectrum. Much evidence favors protein as the absorbing substance because its absorption spectrum agrees very closely with the absorption spectrum of the skin; but Clark (1936) has demonstrated that the latent period for proteins and that for erythema have very different temperature coefficients. This makes it seem improbable that proteins are the sole factor in absorption. Additional investigations have also demonstrated the absorbing power of nucleic acids, but their absorption spectra markedly differ from the absorption spectrum of the

skin. However, since nucleic acids and proteins are both very important cellular constituents further study might reveal that there is some interaction of the two which ultimately reveals itself in the appearance of erythema.

The cells which carry on absorption finally release a substance which, in turn, causes the dilation of the papillary blood vessels. It is this dilation which increases the flow of blood into the cutaneous areas and results in the characteristic redness of the skin common to erythema. The substance which the cells release to cause the dilation is still much in doubt. Many authors have ascribed to it a histamine-like nature resulting from the break-down of histidine in the cells. But numerous other investigators have not been in complete accord with this theory so that just what this substance is that causes dilation of the cutaneous blood vessels is still in doubt.

The normal sequel to erythema is pigmentation of the skin. It has been generally agreed that this process is brought about by the conversion of tyrosine to dihydroxyphenylalanine (dopa) by the enzyme tyrosinase and this, in turn, is converted to melanin. The production of melanin occurs in the basal cell layer and it later moves into the superficial layers of the epidermis where it manifests itself as a visible tan. However, not all pigmentation is due to the formation of melanin as it has been found that ultraviolet wave-lengths, longer than those which cause erythema and melanin production, are capable of changing a reduced form of melanin into oxidized melanin.

The development of protection against sunburn is the result

of two processes: (a) the thickening of the corneum which decreases the transmission of the ultraviolet rays into the deeper layers of the epidermis, and (b) the formation of pigment. Both protective mechanisms are able to absorb ultraviolet to a great degree; but the first is much more effective than the second because the pigment is located in the deeper layers of the epidermis and, hence, does not absorb rays until they have already penetrated to the living cells.

It is difficult to establish a normal erythemal threshold for sunburn because every individual varies in his response to irradiations. Although the erythemal action spectrum is the same for all, the intensity and duration of exposure required to produce a reaction varies from one individual to another. These variations are due to a great number of physical and physiological factors in each individual and furthermore, a given person may differ in his threshold from time to time. There are also many factors which cause the radiations themselves to differ, such as location, smoke, fog, wind, and many other variables in the atmosphere and on the ground.

Ultraviolet has a very beneficial antirachitic action. The presence of 7-dehydrocholesterol, which isthe precursor of vitamin D, in the most superficial layers of the skin absorbs radiations very strongly around 2,800 A.U. and, as a result, is converted into vitamin D.

Cutaneous cancer may be induced by radiations ranging from 2,900 to 3,200 A.U. The agent which causes the manifestation of skin cancer hasyet to be determined, but it does undoubtedly fol-

low some kind of cell injury that gives rise to an increased mitosis of the cells in the irradiated region. Much work is being done on this at present. Once the cause of the abnormal cellular poliferation is understood, it may possibly reveal the mystery of other types of cancer to science, and result in the release of mankind from one of his greatest scourges.

A B S T R A C T

The spectral range of light from 2,400 A.U. to 4,400 A.U. is the region in which ultraviolet light is located. Normal human skin is capable of many reactions to light radiations in this range; and it is with these various reactions that this paper is most concerned.

Skin serves as a very effective filter in determining to what degree the various ultraviolet wave-lengths will penetrate into the deeper parts of the body. On the whole, a layer of epidermis only 0.1 mm. thick has the power to absorb almost all of the radiations falling on the skin, and thus protect the deeper cells from any injury which the ultraviolet may cause. The distance that these waves penetrate into the skin is dependent on the part of the spectrum in which they occur, since different layers of the skin show characteristicabsorption bands for different spectral lines. The greatest amount of absorption takes place around 2,800 A.U. where the stratum corneum and stratum granulosum absorb nearly all of the waves in this region. As a result, almost no reactions occur at this spectral line because the rays are unable to penetrate to the living cells and cause any changes therein. On each side of 2,800 A.U., the penetrating power of the ultraviolet waves increases until the waves are able to reach the lower layers of the epidermis where the living cells are found and, thus, cause photobiologic reactions to occur. The greatest degree of penetration is around 2,950 A.U. where these rays are able to reach the basal cell layer

and cause the normal skin responses of erythema accompanying sunburn and followed by a suntan. These rays have a direct effect on the prickle cells of the stratum spinosum, resulting in their destruction and the elaboration of certain substances which cause the visible manifestation of erythema and pigmentation. These processes exhibit their strongest reactions at 2,970 A.U. with a second smaller maximum at 2,500 A.U. Below 2,400 A.U. absorption by the superficial horny layers is so great that no rays can penetrate to the living cells to cause any reactions.

The appearance of sunburn and suntan does not immediately take place following exposure to the ultraviolet radiations. There is a definite delay known as the latent period during which various reactions in the cells are carried on to form new substances which are liberated from these cells and, in turn, affect other body systems which ultimately cause the appearance of the burn andtan. The latent period is made up of two distinct parts: the first part is the time following the exposure during which intracellular changes are going on characterized by a temperature coefficient of one; the second part is the time which lapses between the release of the new substances from the cell and the appearance of a just perceptible erythema characterized by a temperature coefficient of 2.3. This latent period presents a considerable problem in understanding the internal mechanism which governs the appearance of the erythema. The first part of the period, with its temperature coefficient of one, corresponds to many substances that are essentially protein in

nature which also have a temperature coefficient of one when they are denaturated by ultraviolet radiations. However, the temperature coefficient of 2.3 in erythema production has no counterpart in protein denaturation which has a temperature coefficient of eight to ten in the time which corresponds to the latent period of erythema. For this reason it is rather difficult to draw a parallel between the appearance of erythema and protein coagulation. The exact nature of the latent period is still unknown, as well as the events during this interval in the cells.

Since protein denaturation has a temperature coefficient of eight to ten during its latent period, whereas the latent period preceding the formation of erythema has a temperature coefficient of 2.3, it seems highly improbable that the absorbing substance in the cell, which initiates the erythemic response, is a pure protein. Although the absorption spectrum ofproteins ismuch the same as the absorption spectrum of the skin, this difference in the temperature coefficients makes it necessary to seek out some other substance which may also react to ultraviolet radiations. Nucleic acids have been demonstrated to have a great absorbing power within the erythemic action spectrum. The main difference between absorption of the nucleic acids and that of the skin is that the maximum for the nucleic acid absorption spectrum is at 2,600 A.U. while the maximum for the skin absorption spectrum is 2,800 A.U., which isthe same as the absorption maximum of proteins. Despite this difference it must be remembered that nucleic acids, as well as proteins, are important cellular constituents and it is very possible that when the true

nature of the absorbing substance is worked out, it will be found that the response which initiates erythema is the result of an interaction between proteins and nucleic acids.

Following the absorption of the ultraviolet radiations by the absorbing substance of the prickle cells, a degeneration of these cells occurs which liberates a product that has the power to cause the dilation of the cutaneous blood vessels in the papillary layer of the corium. This dilation increases the amount of blood flowing through the skin; and the excess blood flow manifests itself as a reddening of the skin, which is popularly known as sunburn. Many investigators believe that this substance released from the degenerate prickle cells results from the breakdown of the protein histidine to form a histamine-like substance. When histidine is irradiated in vitro it forms a product that has many characteristics like histamine; and when this histamine-like substance ("H" substance) is injected into the skin, it causes a typical erythemal response to appear. However, many other studies have discredited the "H" substance theory to a considerable extent. Chemical analysis has revealed that the break-down product of irradiated histidine does not react to various tests the same way that pure histamine does. In addition, although this "H" substance causes an erythema to appear when injected into the skin, it is now followed by pigmentation such as in erythema formed by exposure to ultraviolet. The histidine-histamine reaction requires the presence of oxygen for its completion; and it has been found that the erythema of sunburn can occur in the absence of oxygen. The factor of

the latent period also presents a serious objection to this theory for it is inconceivable that a histidine molecule will retain its activation power only to complete the reaction at a later time. For these reasons, there is a considerable amount of doubt concerning the existence of an "H" substance; and this aspect of photobiologic processes, like some of those already presented, still merits a great deal of study as to what the substance is that causes the dilation of the cutaneous blood vessels.

Several days after the appearance of erythema, pigmentation begins to form in the skin as a normal sequel to sunburn. The process by which thisoccurs has been well established and has been generally agreed upon by most investigators. Suntan results from the conversion of tyrosine to 3-4 dihydroxyphenylalanine (dopa) by the enzyme tyrosinase; and the dopa, in turn, is converted to melanin by the enzyme dopaoxidase. These reactions take place in the dendritic melanoblasts of the basal cell layer; and then the cells with the pigment migrate to the superficial layers of the epidermis, producing the appearance known as suntan. This phase of pigment formation results from ultraviolet radiations in the same spectral region as the erythemal action spectrum (2,400 to 3,250 A.U.). However, there is another type of pigmentation which results from exposures ranging from 3,000 to 4,400 A.U. with a broad maximum at 3,400 A.U. This second type of pigmentation is not preceded by erythema, as the first type is; and it also does not cause as deep a tanning as the first kind. This process has been termed "darkening of the preformed pig-

ment" because it is not the result of the formation of new melanin but is brought about by changing a reduced form of melanin, already present in the basal cell layer, into oxidized melanin. When both types of pigmentation occur at the same time, the skin has a deeper bronze color than when either occurs alone.

Protection of the skin from ultraviolet radiations and future sunburn is not due mainly to pigment formation as is commonly believed. Most protection is the result of a thickening of the corneum which increases absorption and, thus, decreases the amount of ultraviolet light which isable to penetrate into the deeper layers of the epidermis. There are several reasons why thickening of the corneum is a better protective device than pigmentation, although melanin also is a very effective absorbing agent of ultraviolet rays. In the first place, thickening takes place only a few hours after exposure; and, thus, its protective action is manifested long before pigmentation appears, which usually takes several days. Secondly, the pigment is found below the point where cell destruction occurs, since the pigment is in the basal cell layer, whereas cell destruction occurs above this layer in the prickle cells. The thickening of the corneum takes place in the most superficial layers and protects all cells below it. Finally, the thickening of the cornium disappears many months before pigmentation and the skin assumes its normal response to ultraviolet radiations while pigment is still present in the skin. However, all of these factors do not rule out the importance of melanin, for its location in the basal cell

layer prevents rays that do penetrate that far from going any further. The only effectiveness in artificial protective devices is their ability to increase scattering of the ultraviolet rays. Protective ability varies among individuals and depends in part upon the thickness of the layer of oil that the people apply to the skin. The appearance of erythema may also be inhibited by the direct action of long ultraviolet rays on the cutaneous vessels of the papillary layer where they inhibit the dilation of these blood vessels by altering their sensitivity to the dilator substances released by the damaged prickle cells.

However, all individuals do not react in the same way to the erythemic action spectrum although this spectrum is a constant. The intensity and duration of the exposures required to produce erythema vary considerably. Thus it is practically impossible to establish a standard erythemal threshold for the population. These variations are the result of many physical and physiological factors inherent in each individual which manifest themselves, in one way, by effecting the person's sensitivity to ultraviolet radiations. Since every person has a different body constitution, it naturally follows that everyone will differ in their degree of sensitivity. The erythema threshold can also vary in any given person from time to time for similar reasons. The intensities of radiations in sunlight are never constant and many factors such as wind, smoke, fog, snow, and many other variables both in the air and on the ground can change the intensity of the ultraviolet light in the atmosphere.

Besides causing erythema and pigmentation, ultraviolet light has a very beneficial action in preventing and curing rickets. There is found in the most superficial layers of the skin, the precursor of vitamin D, 7-dehydrocholesterol, which absorbs ultraviolet radiations very strongly in the region of 2,800 A.U. This process is not an adjunct to erythema and pigmentation because the action spectrum for erythema and pigmentation is at a decided minimum around 2,800 A.U., whereas the action spectrum for the converion of 7-dehydrocholesterol to vitamin D is at its greatest maximum around 2,800 A.U. The fact that this process occurs in the same spectral range as the production of erythema and pigmentation is nothing more than a coincidence.

One seriouseffect of ultraviolet radiation on the skin is its ability to increase mitosis of the cells to such a degree that it may cause cutaneous cancer. This first became evident when various investigators noted that skin cancer is most prevalent on those parts of the body (95% on the face and hands) which are constantly exposed to sunlight and occurs most frequently in people who are continually working in the sun. Subsequent investigations have shown that the action spectrum lies in the ultraviolet range of 2,900 to 3,200 A.U. Experiments on mice and rats have conclusively demonstrated that these wave-lengths are capable of inducing sarcomas in these animals. However, caution must be used in interpreting these results as far as human cutaneous cancer is concerned, because those tumors most prominent in humans are carcinomas. Nevertheless, the evidence clearly indicates that it is the ultraviolet

part of the sunlight spectrum which is responsible for the appearance of cutaneous cancers. The manner in which these radiations affect the cells to cause an increase in mitosis is still not known. This problem is currently the subject of very intensive research by the National Cancer Foundation, and its answer will undoubtedly pave the way to a better understanding and possible cure of other types of cancer.

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